Chapter 5

5. A. Scanning Electron Microscopic Study of Erythrocyte Membrane in Albino Mice

INTRODUCTION

The widespread environmental pollution caused by the chemical substances (like pesticides) is a serious problem for all living creatures including human (Mc Clune et. al., 2001; Mc Kinlay et. al., 2008; Boobis et. al., 2008). The number of sources of pesticides in our environment is so high that it is very difficult to avoid exposure to it. Pesticides are sprayed in the vegetable growing areas rice field, horticultural field, home garden, tea garden etc. Several studies have been reported on the effect of pesticides on the aquatic fauna, higher animals and mankind (Lone and Javaid, 1976; Banuffaldi and Cucci, 1989; Agarwal et. al., 1990). The different types of chemical products found in the market which are applied in almost everywhere. Pesticides are also used for timber preservations against termites and in building materials. The absorption of pesticides usually takes place by different ways, such as by inhalation by skin contamination through air, water and dust, soil etc.
REVIEW OF LITERATURE

Across the globe pesticides have been found in human blood, urine, breast milk, semen, adipose tissue, amniotic fluid, infant meconium and umbilical cord blood. Cumulative exposure to pesticides may come from flood, water, air, dust, soil etc. Pesticides can be absorbed though skin contact, inhalation or accidental ingestion. Farm workers come in direct contact with pesticides at work as well and are occupationally exposed to them. When a person is exposed to pesticides, body’s detoxification mechanisms are activated. Some pesticides are metabolized into different chemicals and excreted and some are stored in fatty tissues in the body. Body burden data from analysis of blood provides evidence of exposure to chemicals stored in our body. There are several studies international and national on pesticide residues found in blood samples (Mathur et. al., 2005).

Erythrocytes which are responsible for transport of \( \text{O}_2 \) and \( \text{CO}_2 \) have complex mechanising to the studies revealed a damaged erythrocytes membrane in the albino-mice. A similar kind of surface damage followed by increased permeability of membrane lending to haemolysis was recorded in RBC of higher animal including human being when exposed to various classes of pesticide (Agrawal et. al., 1990). Pesticides causes change in the shape of albino-mice erythrocytes because maximum number of pesticides are highly lipid soluble. As a result it alters the phospholipids of plasma membrane and consequently RBC’s shape (Banuffaldi and Cucchi, 1989).
The study can be assumed that pesticides contamination (through air, water or soil by inhalation or dermal) absorbed by the organisms are transported through blood to different tissue systems (Allen, 1995) which affect is their RBC configuration and function maintained by membrane integrity, which is quite flexible and keep haemoglobin functional. The study is an attempt to determine the extent of deformation undergone of RBC of albino-mice with pesticide treatment in two different time intervals in different concentrations.

MATERIALS AND METHODS

The experiment was carried out to observe the effect of pesticides on the red blood cells of albino mice. Swiss albino mice originally obtained from Pasture Institute, Shillong, India. The animals were housed in polypropylene cages (10 animals per cage with Sawdust as the bedding material) at 25±5°C temperature on a 12h light/dark cycle. The animals were supplied with dry food pellets commercially available (Hindustan Liver Ltd., New Delhi) and water. Healthy male animals in the age group of 10-12 weeks and weighting 20-25 g were used as test animals.

Scanning Electron Microscopy

Fixation was achieved by placing a drop of blood in 0.1 m 2% glutanaldehyde buffered with sodium cacodylate for 30 min. The sample was centrifuged at 1500 rpm for 5 min, washed and resuspended in distilled water, and the process was repeated two or three times. A thin film was
decanted and applied to a coverslip after resuspending in distilled water, and air dried. After air dry the samples were studied under Scanning Electron Microscope at the sophisticated analytical instrumentation facilities, North East Hill University, Shillong. There a complete and conductive coating of gold was applied using a fine coat ion sputten (Jeol, JFC-1100). Observations were made on a scanning electron microscope (Jeol, JSM-6360) at an accelerating voltage of 15-20 kv.

**Chemicals**

Chlorpyriphos \((C_{9}H_{11}Cl_{3}NO_{3}PS)\)

O,O-diethyl O-3,5,6-trichloropyridin-2-y1 phosphorothioate

Chlorpyrifos 20% EC (Kaman) mfd. by Crystal Phosphate Ltd.

Malathion \((C_{10}H_{19}O_{6}PS_{2})\)

[S-1,2-bis(ethoxycarbonyl) ethyl O, O-diethyl phosphorodithioate]

Malathion (Kunamala 50) 50% EC, Kundu Agrochemical Pvt. Ltd.

Endosulfan \((C_{9}H_{6}Cl_{6}O_{3}S)\)

Endosulfan (Thiodan) 35%, Bayer Crop Science Ltd.

1,4,5,6,7,7-Hexachloro-8,9,10-trinorborn-5-en-2,3-ylenebismethylene) sulfite

Cypermethrin \((C_{22}H_{22}ClNO_{3})\)

[Cyano (3-phenoxyphenyl) methyl - 3- (2, 2-dichloro-ethenyl), 2, 2-dimethylcyclopropanecanboxylate]

Cypermethrin (K-HERO) 10%EC, Kundu Agrochemical (P) Ltd.

Fenvalerate \((C_{25}H_{20}ClNO_{3})\)

α-Cyano-3-phenoxybenzyl-(s)-2-(4-chlorophenyl)-3-Methylbutyrate

Fenvalerate 20% EC, Rallis India Ltd., Agrochemicals Division
For the experiment five types of pesticides were selected from different chemical groups i.e., malathion, cypermethrin, endosulfan, chlorpyriphos, fenvalerate for albino mice. Three different doses were selected for the experiment with concentrations of 5mg/kg, 10mg/kg and PSVT (pesticides sprayed vegetables treatment) and control groups received normal water. After 30 days and 60 days of above treatments, the blood taken from the albino mice and slides were prepared for scanning electron microscopic study.

**RESULT**

The results of the above experiments are described below.

**Discocytes-echinocytes transformation**

The most significant change in the erythrocytes membrane due to pesticides toxicity was in the transformation of discocytes to echinocytes. The biconcave shape of the discocyte was changed into a sphere converted with crenations or spicules (fig. 1). All the experimental groups the discocytes–echinocytes transformation was higher. Various forms of the deformed discocytes (echinocytes transformation) were seen in all the groups of albinomice exposed to pesticides. The pesticides sprayed vegetables treated group of albinomice the discocytes-echinocytes transformation were also found.
**Stomatocytogenesis**

In addition to discocytes echinocyte transformation, the other pronounced change in the erythrocyte membrane occurred in the form of development of stomatocytes. The membrane was folded one side as shown in fig. 2. The normal central depression, as observed in discocytes, appeared almost like a hole in the centre of the cell, and biconcave shape of the cell was completely lost. This type of transformed cell is termed a stomatocytes (Barnhart *et al.*, 1978; Doll and Allenden 1978), and they have been reported in various haemolytic diseases.

**Formation of spherocytes**

In addition to echinocytes and stomatocytes, the appearance of spherocytes was evident in all the experimental groups of mice exposed to pesticides.

In the formation of spherocytes, the disc-shaped RBCs were transformed into spheres. They were oval to round in shape and appeared dense in comparison with the normal red cells. They were swollen RBC cells, in extreme cases the membrane were ruptured (Fig. 3). Spherocytosis is characterised by presence of spheroidal rather than biconcave disc-shaped red cells. Spherocytes are seen in autoimmune haemolytic anaemia and in ABO haemolytic disease of the albino-mice.
Membrane internalization and wrinkling

All the experimental groups of albino mice showed various forms of membrane internalization or wrinkling of RBC. This type of erythrocyte modification including uniform internal in foldings of membrane and a longer longitudinal infolding of the membranes, leading to shrinking of the cell (Fig. 4).

Appearance of ring-shaped cells

A few cells observed in the blood smears of experimental groups had shaped and morphologically similar to a ring. The characteristic feature of the cell was the presence of alternate bands of electron light and electron dense areas (Fig 5)

Appearance of Tear-drops shaped cells

A few cells observed in the blood smears of experimental groups had shape and morphology similar to a tear-drop. The characteristic features of cells were the pointed in one edge and the other edge was round (Fig. 6).

Occurrence of Reticulocytes

A few cells, of the RBC population were found to undergo reticulocytogenesis. The reticulocytes were characterised by the presence of networks of membranes and thin size were reduced in comparison to normal discocytes (fig. 7).
Erythrocytes carry haemoglobin (the molecules that transport oxygen) and help remove wastes from tissues throughout the body (Gale, 2008).

The study is an attempt to determine the extent of deformation undergone by RBC of albino mice inhabiting with pesticide treatment of two different time duration in different concentrations.

**Appearance of spindle shaped cells**

A few cell observed in the blood smears of experimental groups of albino mice with hand shaped and morphology similar to a spindle. The characteristic features of the cell was the pointed shape in both edges and swollen middle portion.

The results of the experiment are described above, such as echinocytes, deformed discocytes, membrane internalization, spherocytes, reticulocytes, spindle shaped, tear drops etc. These all deformatives are found in various concentrations with two time durations of these pesticides. So, the results are shown in the figure 8.
Fig. 1 Discocytes-echinocytes
Fig. 2 Stomatocytogenesis
Fig. 3 Formation of spherocytes
Fig. 4 Membrane internalization
Fig. 5 Ring-shaped cells
Fig. 6 Tear-drops shaped cells
Fig. 7 Reticulocytes
Fig. 8 Spindle shaped cells
Table 1: Types of abnormalities observed in RBC of albino-mice under different pesticide treatments (5 mg/kg body wt/30 days)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endosulfan</td>
<td>Echinocytes, Deformed-discocytes, Membrane internalization, Spherocytes.</td>
</tr>
<tr>
<td>Chlorpyriphos</td>
<td>Deformed-discocytes, Echinocytes, Membrane internalization, Spherocytes, Stomatocytes</td>
</tr>
<tr>
<td>Malathion</td>
<td>Membrane internalization, Echinocytes, Deformed-discocytes, Spherocytes.</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>Echinocytes, Deformed-discocytes, Membrane internalization, Spherocytes.</td>
</tr>
<tr>
<td>Fenvalerate</td>
<td>Reticulocyte, Deformed-discocytes, Membrane internalization, Echinocytes, Spherocytes, Stomatocytes.</td>
</tr>
<tr>
<td>Control</td>
<td>No abnormalities</td>
</tr>
</tbody>
</table>

Table 2: Types of abnormalities observed in RBC of albino-mice under different pesticide treatments (10 mg/kg body wt/30 days)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endosulfan</td>
<td>Membrane internalization, Echinocytes, Deformed-discocytes, Spindle Shaped cell, Stomatocytes, Spherocytes, Tear drop shaped cell.</td>
</tr>
<tr>
<td>Chlorpyriphos</td>
<td>Deformed-discocytes, Echinocytes, Spherocytes, Membrane internalization, Reticulocytes.</td>
</tr>
<tr>
<td>Malathion</td>
<td>Echinocytes, Spherocytes, Stomatocytes, Deformed-discocytes, Membrane internalization</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>Echinocytes, Tear drop shaped cell, Spherocytes, Spindle shaped cell, Deformed-discocytes, Membrane internalization, Stomatocytes.</td>
</tr>
<tr>
<td>Fenvalerate</td>
<td>Membrane internalization, Echinocytes, Spherocytes, Stomatocytes, Spindle shaped cell, Deformed-discocytes.</td>
</tr>
<tr>
<td>Control</td>
<td>No abnormalities</td>
</tr>
</tbody>
</table>
### Table 3: Types of abnormalities observed in RBC of albino-mice under Pesticide Sprayed Vegetables Treatment (PSVT/kg body wt/ 30 days)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endosulfan</td>
<td>Echinocytes, Membrane internalization, Deformed-discocytes, Stomatocytes, Spherocytes, Tear drop shaped cell.</td>
</tr>
<tr>
<td>Chlorpyriphos</td>
<td>Echinocytes, Membrane internalization, Deformed-discocytes.</td>
</tr>
<tr>
<td>Malathion</td>
<td>Membrane internalization, Deformed-discocytes, Stomatocytes, Spherocytes.</td>
</tr>
<tr>
<td>Cypermithrin</td>
<td>Spherocytes, Membrane internalization, Deformed-discocytes, Tear drop shaped cell, Echinocytes.</td>
</tr>
<tr>
<td>Fenvalerate</td>
<td>Stomatocytes, Spherocytes, Membrane internalization, Deformed-discocytes, Tear drop shaped.</td>
</tr>
<tr>
<td>Control</td>
<td>No abnormalities</td>
</tr>
</tbody>
</table>

### Table 4: Types of abnormalities observed in RBC of albino-mice under different pesticide treatments (5 mg/ kg body wt/ 60 days)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endosulfan</td>
<td>Echinocytes, Deformed-discocytes, Membrane internalization, Spherocytes.</td>
</tr>
<tr>
<td>Chlorpyriphos</td>
<td>Membrane internalization, Deformed-discocytes, Stomatocytes, Spherocytes</td>
</tr>
<tr>
<td>Malathion</td>
<td>Echinocytes, Membrane internalization, Deformed-discocytes, Spherocytes, Reticulocyte, Stomatocytes.</td>
</tr>
<tr>
<td>Cypermithrin</td>
<td>Echinocytes, Membrane internalization, Deformed-discocytes, Spherocytes.</td>
</tr>
<tr>
<td>Fenvalerate</td>
<td>Deformed-discocytes, Spherocytes, Echinocytes, Membrane internalization, Stomatocytes.</td>
</tr>
<tr>
<td>Control</td>
<td>No abnormalities</td>
</tr>
</tbody>
</table>
### Table 5: Types of abnormalities observed in RBC of albino-mice in different pesticide treatments (10 mg/kg body wt/60 days)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endosulfan</td>
<td>Membrane, internalization, Echinocytes, Deformed-discocytes, Stomatocytes, Spherocytes, Reticulongties.</td>
</tr>
<tr>
<td>Chlorpyriphos</td>
<td>Spherocytes, Echinocytes, Membrane, internalization, Tear drop shaped cell, Deformed-discocytes, Spindle shaped cell.</td>
</tr>
<tr>
<td>Malathion</td>
<td>Echinocytes, Membrane internalization, Deformed-discocytes, Spherocytes, Spindle shaped cell, Stomatocytes.</td>
</tr>
<tr>
<td>Cypermithrin</td>
<td>Tear drop shaped cell, Echinocytes, Membrane internalization, Deformed-discocytes, Stomatocytes.</td>
</tr>
<tr>
<td>Fenvalerate</td>
<td>Echinocytes, Membrane internalization, Deformed-discocytes, Stomatocytes, Spherocytes, Spindle shaped cell.</td>
</tr>
<tr>
<td>Control</td>
<td>No abnormalities</td>
</tr>
</tbody>
</table>

### Table 6: Types of abnormalities observed in RBC of albino-mice under Pesticides Sprayed Vegetables Treatment (PSVT/kg body wt/60 days)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endosulfan</td>
<td>Echinocytes, Stomatocytes, Spherocytes, Membrane internalization, Deformed-discocytes, Tear drop shaped cell.</td>
</tr>
<tr>
<td>Chlorpyriphos</td>
<td>Stomatocytes, Spherocytes, Membrane internalization, Deformed-discocytes, Echinocytes.</td>
</tr>
<tr>
<td>Malathion</td>
<td>Tear drop shaped, Stomatocytes, Spherocytes, Membrane internalization, Deformed-discocytes, Echinocytes.</td>
</tr>
<tr>
<td>Cypermithrin</td>
<td>Tear drop shaped, Echinocytes, Stomatocytes, Spherocytes, Membrane internalization, Deformed-discocytes.</td>
</tr>
<tr>
<td>Fenvalerate</td>
<td>Spherocytes, Membrane internalization, Deformed-discocytes, Echinocytes, Tear drop shaped, Ring Shaped cell.</td>
</tr>
<tr>
<td>Control</td>
<td>No abnormalities found</td>
</tr>
</tbody>
</table>
DISCUSSION

Scanning electron microscopy revealed that various forms of discocyte-echinocyte transformation, stomatocyte-spherocytic changes, membrane internalization, and reticulocytogenic effects were caused by pesticides toxicity in the erythrocytes of different groups of experimental albino mice. The information obtained on membrane changes in the RBC may be useful in understanding the haematological problems which result from lead toxicity. It is well known that the abnormalities in cell shape of erythrocytes is of considerable pathological importance, and that a delicate equilibrium of extrinsic and intrinsic forces determined the shape of an erythrocyte (Bessis and Weed, 1972). Thus the correct interpretation of the fine details of RBC can yield valuable information on the physiopathological state of the cell.

Changes in blood characteristics resulting from the accumulation of pesticides occur fairly early. It has been established that pesticides inhibits the enzymes which are involved in incorporation of iron into the protoporphyrin molecule to form haeme.

In blood, the target of toxicity for a variety of sparingly soluble toxicants is the erythrocyte membrane, which is frequently used for the study of the interaction of xenobiotics with biomembranes. All disordered red blood corpuscles (RBC) manifest some time to loss of membrane plasticity, which greatly impairs RBC movement through the microvasculature (Young et. al. 1951; Barnhart and Lusher, 1976). The
slightest damage to this membrane can cause haemolysis, resulting in constituents leaking out of the corpuscles.

It is notable that the degree of membrane response to different extrinsic factors or to the same factor varies according to the physiopathological state of individual cells (Barnhart *et. al.* 1978), and also to the concentration and chemical nature of the toxicant. Furthermore, it is known that a significant proportion of toxic substances absorbed in the body during pregnancy accumulate in the developing embryo, and affects the offspring.

Light microscopy with a limited resolution, and transmission electron microscopy dedicated to revealing only the detailed internal structure, cannot fully analyse the fine details of the three-dimensional shape of the cell. Scanning electron microscopy (SEM), because of its considerable depth of field and high resolving power has been found to be the best technique for studying the structural detail of normal and pathological erythrocytes (Salsbury and Clarke, 1967; Bessis and Lessin, 1970; Bessis and Dobler, 1970; Van Oss and Mohn, 1970).

The first application of SEM to pathological RBC was made by Salsbury and Clarke (1967). Later several investigators used SEM in order to study the membrane dynamics of erythrocytes, and found the instrument to be instructive in denoting individual variations in both functionally stressed, and the initial population of RBC.
To maintain haemoglobin function, the erythrocyte has a complex mechanism to secure the integrity of its membrane (Barnhart et al. 1978). Various congenital and acquired disorders affect the mechanism thereby disturbing the membrane integrity. This results in impairing the function of the erythrocyte cells and also affects their survival.

Paroxysmal nocturnal haemoglobinuria (PHN), for example, is an acquired haemolytic disorder which produces abnormalities in the erythrocyte cell membranes, accompanied by increased sensitivity of RBC to lysis by complement. The percentage of membrane abnormalities which result in abnormal erythrocyte shapes was related to the degree of haemolysis. The majority of the cells were observed to have biconcave discs, when haemolysis was minimal; a few cells showed deformities in shape at mild haemolysis; and many of the erythrocytes had various types of morphological abnormalities during severe and continuous haemolysis (Doll and Allender, 1978). Various forms of stomatocyte-spherocyte and discocyte-echinocyte transformations were evident in this disorder. The different types of abnormalities of the erythrocyte membrane which result in deformed shapes of the cells have also been reported in patients with iron deficiency, post-splenectomy, hereditary spherocytosis, and autoimmune haemolytic anaemia.

In the present work, the high percentage of cells showing Membrane internalization, Deformed discoytes, Ectinoceytes, sphenocytes,
stomatocytes in RBC of albino mice of different treatment with pesticides induced toxicity suggests that the pesticide caused haematological problems which might have havoc with human and other life form. The appearance of a high percentage of echinoagtes and deformed discocytes, membrane internalization, spherocytes, stomatocytes and few retionoclocyte, ring shaped cell, tear drops cell, spindle shaped cell in individuals exposed to pesticides toxicity. So, it is clear that pesticide may acts as an echincytogenic, stomatogenic, sphenocytogenic, retinlongtogenic agent for different treatment of pesticides and pesticides sprayed vegetable treatments.

It is of interest to mention that several organic and inorganic compounds have been reported to cause membrane abnormality and shape change in erythrocytes, the degree of which varied with their concentration and chemical nature (Barnhart et al. 1978). Tetrodoxin, for example, was shown to be a stomatocytogenic and an echinocytogenic agent. At $10^{-3}$ M concentrations both stomatocytes and echinocytes evolved in addition to spherocytes, while the response was chiefly stomatocytogenic from $10^{-4}$ to $10^{-8}$ M. Calcium ionosphere A 23187 at concentrations of $10^{-3}$ to $10^{-5}$ M converted normal RBC into echinocytes (White, 1974, 1976; Barnhat et al. 1978). Vincristine and vinblastine affected normal RBC only when the concentrations of the compounds exceeded $10^{-5}$ M, and the number of stomatocytes and spherocytes increased. Sodium chloride, at a concentration
of 0.6% changed the shape of RBC significantly, whilst discocyte asymmetry increased from 18 to 37%, and the number of spherocytes increased (Barnhart et al. 1978). However, the degree of haemolysis was low in this case.

All these examples and observations on the effect of pesticides on erythrocyte membrane of different groups of experimental albino mice, suggests that pesticide acts as an echinocytogenic and stomatocytogenic agent, and causes various degrees of haemolysis which is reflected in the abnormally shaped of erythrocytes. Membrane internalization of erythrocytes observed in our work appeared to be related to a higher degree of haemolysis (Barnhart et al. 1978). Ring-shaped cells indicated a decreased cellular haemoglobin concentration (Bessis and Weed, 1972). The presence of reticulocytes was also indicative of haemolysis, since the reticulocyte number can be extremely high in haemolytic anaemia (Barnhart et al. 1978).

The normal deformability caused by certain echinocytogenic agents such as calcium ionophore A 23187 has been shown to be governed by the intramembranous calcium pump powered by ATP (Weed, 1975; Weed and Chailley, 1973). The stomatocytogenic and spherocytogenic effect of vincristine and vinblastine have been observed to be due to their interference with phosphorylation (Barnhart et al. 1978). Presumably protein kinase is inhibited, and protein microfilaments are denatured or
precipitated. This affects the contractility and deformability of the RBC (Marantz et. al., 1969).

Since it is known that pesticides binds to mitochondrial membranes and suppresses both oxidative phosphorylation and citric acid cycle (Friberg et. al. 1986), the changes in the erythrocyte membrane of albino mice due to the action of pesticides can be explained in terms of its interference with phosphorylation, inhibition of protein kinase and denaturation of protein microfilaments.

Furthermore, it is known that toxicants, by damaging the cell membrane, allow free passage of ions and water between the interior and the exterior of the cell. Due to the entry of water into the cell, it undergoes swelling, eventually becomes a spherocyte and finally ruptures (Dourmashkin and Ross, 1966). The swelling of cells and rupturing of the cell membrane were prominent in experimental groups in the present study. This suggested the involvement of pesticides as a strong spherocytogenic agent when exposure to it was severe.

However, the appearance of different types of membrane abnormalities in albino mice induced toxicity with pesticides is most interesting. Thus, the mechanism by which the element affects erythrocytes during their development and whether the changes observed in the albino mice are permanent or reversible requires further elucidation.
Fig. 24: RBC of albino mice showing abnormalities in Endosulfan pesticides (5 mg/kg body wt/day/60 days).

a: Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI) showing pesticide toxicity. X 3,500

b: Stomatocyte (ST), Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI) showing pesticide toxicity. X 2,700

c: Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI) showing pesticide toxicity. X 6,000

d: Echinocyte (E), Deformed-discocyte (DD), Spherocyte (SP) showing pesticide toxicity. X 3,000
**Fig. 25**: RBC of albino mice showing abnormalities in Endosulfan pesticides (10 mg/kg body wt/day/60 days).

- **a**: Echinocyte (E), Stomatocyte (ST), Deformed-discocyte (DD), Membrane internalization (MI), Spherocyte (SP) showing pesticide toxicity. X 2,700
- **b**: Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI) showing pesticide toxicity. X 2,700
- **c**: Echinocyte (E), Deformed-discocyte (DD), Reticulocyte (R), Membrane internalization (MI) showing pesticide toxicity. X 3,000
- **d**: Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI), Stomatocyte (ST) showing pesticide toxicity. X 3,300
- **e**: Echinocyte (E), Deformed-discocyte (DD), Spherocyte (SP) Membrane internalization (MI) showing pesticide toxicity. X 3,700
**Fig. 26**: RBC of albino mice showing abnormalities in Endosulfan pesticides (PSVT/kg body wt/day/60 days).

a: Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI) showing pesticide toxicity. X 3,000

b: Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI), Spherocyte (SP) showing pesticide toxicity. X 3,000

c: Deformed-discocyte (DD), Spherocyte (SP), Stomatocyte (ST) showing pesticide toxicity. X 3,000

d: Deformed-discocyte (DD), Membrane internalization (MI), Spherocyte (SP) showing pesticide toxicity. X 3,500
Fig. 27: RBC of albino mice showing abnormalities in Chlorpyriphos pesticides (5 mg/kg body wt/day/60 days).

a: Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI) showing pesticide toxicity. X 2,500

b: Stomatocyte (ST), Tear Drop (TD) showing pesticide toxicity. X 4,300

c: Echinocyte (E), Deformed-discocyte (DD), Spherocyte (SP) showing pesticide toxicity. X 4,500

d: Echinocyte (E), Deformed-discocyte (DD), Spherocyte (SP) showing pesticide toxicity. X 2,700

e: Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI) showing pesticide toxicity. X 1,900
Fig. 28: RBC of albino mice showing abnormalities in Chlorpyrifos pesticides (10 mg/kg body wt/day/60 days).

a: Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI) showing pesticide toxicity. X 2,700
b: Tear Drop (TD) showing pesticide toxicity. X 9,000
c: Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI), Spindle shaped (S) showing pesticide toxicity. X 3,300
d: Echinocyte (E), Deformed-discocyte (DD), Spherocyte (SP), Membrane internalization (MI), Stomatocyte (ST), showing pesticide toxicity. X 2,500
Fig. 29: RBC of albino mice showing abnormalities in Chlorpyriphos pesticides (PSVT/kg body wt/day/60 days).

a: Echinocyte (E), Spherocyte (SP) showing pesticide toxicity. X 3,500
b: Echinocyte (E), Deformed-discocyte (DD), Spherocyte (SP) showing pesticide toxicity. X 2,700
c: Echinocyte (E), Membrane internalization (MI) showing pesticide toxicity. X 2,700
d: Echinocyte (E), Deformed-discocyte (DD), Spherocyte (SP) showing pesticide toxicity. X 3,000
e: Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI), Spherocyte (SP) showing pesticide toxicity. X 3,500
Fig. 30: RBC of albino mice showing abnormalities in Malathion pesticides (5 mg/kg body wt/day/60 days).

a: Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI) showing pesticide toxicity. X 2,500

b: Echinocyte (E), Membrane internalization (MI) showing pesticide toxicity. X 6,500

c: Echinocyte (E), Spherocyte (SP), Deformed-discocyte (DD), Stomatocyte (ST) showing pesticide toxicity. X 4,000

d: Echinocyte (E), Deformed-discocyte (DD), Spherocyte (SP), Membrane internalization (MI) showing pesticide toxicity. X 2,000
Fig. 31: RBC of albino mice showing abnormalities in Malathion pesticides (10 mg/kg body wt/day/60 days).

a: Echinocyte (E), Stomatocyte (ST), Spherocyte (SP), Deformed-discocyte (DD), Membrane internalization (MI) showing pesticide toxicity. X 2,700

b: Echinocyte (E), Deformed-discocyte (DD), Spherocyte (SP) showing pesticide toxicity. X 7,500

c: Echinocyte (E), Deformed-discocyte (DD) showing pesticide toxicity. X 6,000

d: Deformed-discocyte (DD), Spindle shaped (S), Stomatocyte (ST), Membrane internalization (MI), showing pesticide toxicity. X 3,500
Fig. 32 : RBC of albino mice showing abnormalities in Malathion pesticides (PSVT/kg body wt/day/60 days).

a : Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI), Tear Drop (TD) showing pesticide toxicity. X 2,200

b : Deformed-discocyte (DD), Spherocyte (SP), Membrane internalization (MI) showing pesticide toxicity. X 3,000

c : Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI) showing pesticide toxicity. X 2,700

d : Deformed-discocyte (DD), Stomatocyte (ST), Echinocyte (E), showing pesticide toxicity. X 4,500
Fig. 33: RBC of albino mice showing abnormalities in Cypermethrin pesticides (5 mg/kg body wt/day/60 days).

a: Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI), Spherocyte (SP) showing pesticide toxicity. X 2,200

b: Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI), Spherocyte (SP) showing pesticide toxicity. X 3,000

c: Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI) showing pesticide toxicity. X 3,300

d: Deformed-discocyte (DD), Echinocyte (E), Membrane internalization (MI) showing pesticide toxicity. X 3,000
Fig. 34: RBC of albino mice showing abnormalities in Cypermethrin pesticides (10 mg/kg body wt/day/60 days).

a : Tear Drop (TD), Reticulocyte (R) showing pesticide toxicity. X 9,000
b : Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI) showing pesticide toxicity. X 3,500
c : Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI) showing pesticide toxicity. X 3,000
d : Deformed-discocyte (DD), Stomatocyte (ST) showing pesticide toxicity. X 5,500
Fig. 35: RBC of albino mice showing abnormalities in Cypermethrin pesticides (PSVT/kg body wt/day/60 days).

a : Deformed-discocyte (DD), Membrane internalization (MI) showing pesticide toxicity. X 3,000

b : Echinocyte (E), Deformed-discocyte (DD), Spherocyte (SP) showing pesticide toxicity. X 3,000

c : Deformed-discocyte (DD), Stomatocyte (ST), Membrane internalization (MI) showing pesticide toxicity. X 4,300

d : Deformed-discocyte (DD) showing pesticide toxicity. X 6,000
Fig. 36: RBC of albino mice showing abnormalities in Fenvalerate pesticides (5 mg/kg body wt/day/60 days).

a: Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI), Spherocyte (SP) showing pesticide toxicity. X 1,500

b: Deformed-discocyte (DD), Membrane internalization (MI), Spherocyte (SP), Stomatocyte (ST) showing pesticide toxicity. X 2,000

c: Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI) showing pesticide toxicity. X 3,500

d: Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI), Spherocyte (SP) showing pesticide toxicity. X 3,000
Fig. 37: RBC of albino mice showing abnormalities in Fenvalerate pesticides (10 mg/kg body wt/day/60 days).

a: Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI) showing pesticide toxicity. X 2,700

b: Echinocyte (E), Deformed-discocyte (DD) showing pesticide toxicity. X 3,700

c: Echinocyte (E), Deformed-discocyte (DD) showing pesticide toxicity. X 6,000

d: Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI), Spindle shaped (S) showing pesticide toxicity. X 3,500

e: Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI), Stomatocyte (ST) showing pesticide toxicity. X 1,600
Fig. 38: RBC of albino mice showing abnormalities in Fenvalerate pesticides (PSVT/kg body wt/day/60 days).

a: Deformed-discocyte (DD), Ring shaped (RI) showing pesticide toxicity. X3,000
b: Echinocyte (E), Deformed-discocyte (DD), Spherocyte (SP) showing pesticide toxicity. X2,000
c: Tear Drop (TD) showing pesticide toxicity. X4,500
d: Deformed-discocyte (DD), Membrane internalization (MI), Spherocyte (SP) showing pesticide toxicity. X3,300
e: Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI) showing pesticide toxicity. X2,500
Fig. 9: RBC of albino mice showing abnormalities in Endosulfan pesticides (5 mg/kg body wt/day/30 days).

a: Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI), showing pesticide toxicity. X5,000

b: Echinocyte (E), showing pesticide toxicity. X5,000

c: Echinocyte (E), Deformed-discocyte (DD), Spherocyte (SP), Membrane internalization (MI) showing pesticide toxicity. X2,500

d: Deformed-discocyte (DD), Membrane internalization (MI), Spherocyte (SP) showing pesticide toxicity. X2,000
Fig. 10: RBC of albino mice showing abnormalities in Endosulfan pesticides (10mg/kg body wt/day/30 days).

a : Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI), showing pesticide toxicity. X3,300

b : Deformed-discocyte (DD), Membrane internalization (MI), Spindle shaped (S), Stomatocyte (ST) showing pesticide toxicity. X2,500

c : Deformed-discocyte (DD), Spherocyte (SP), Tear Drop (TD) showing pesticide toxicity. X1,800

d : Deformed-discocyte (DD) showing pesticide toxicity. X6,000
**Fig. 11**: RBC of albino mice showing abnormalities in Endosulfan pesticides (PSVT/kg body wt/day/30 days).

a : Deformed-discocyte (DD), Membrane internalization (MI), Stomatocyte (ST) showing pesticide toxicity. X2,500

b : Echinocyte (E), Membrane internalization (MI), Spherocyte (SP), Stomatocyte (ST), Tear Drop (TD) showing pesticide toxicity. X3,300

c : Echinocyte (E), Deformed-discocyte (DD), Stomatocyte (ST) showing pesticide toxicity. X3,000

d : Deformed-discocyte (DD), Membrane internalization (MI), Spherocyte (SP) showing pesticide toxicity. X2,200
Fig. 12: RBC of albino mice showing abnormalities in Chlorpyriphos pesticides (5 mg/kg body wt/day/30 days).

a: Deformed-discocyte (DD), Membrane internalization (MI), Echinocyte (E) showing pesticide toxicity. X2,000

b: Echinocyte (E), Membrane internalization (MI), Spherocyte (SP), Deformed-discocyte (DD) showing pesticide toxicity. X2,700

c: Echinocyte (E), Deformed-discocyte (DD) showing pesticide toxicity. X3,500

d: Membrane internalization (MI), Spherocyte (SP), Stomatocyte (ST) showing pesticide toxicity. X2,700

e: Deformed-discocyte (DD), Echinocyte (E) showing pesticide toxicity. X3,700
Fig. 13: RBC of albino mice showing abnormalities in Chlorpyriphos pesticides (10 mg/kg body wt/day/30 days).

a : Deformed-discocyte (DD), Echinocyte (E), Spherocyte (SP), Stomatocyte (ST) showing pesticide toxicity. X2,700

b : Echinocyte (E), Membrane internalization (MI), Deformed-discocyte (DD), Reticulocyte (R) showing pesticide toxicity. X2,500

c : Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI) showing pesticide toxicity. X6,000

d : Echinocyte (E) showing pesticide toxicity. X3,500

e : Deformed-discocyte (DD), Membrane internalization (MI) showing pesticide toxicity. X2,700
Fig. 14: RBC of albino mice showing abnormalities in Chlorpyriphos pesticides (PSVT/kg body wt/day/30 days).

a: Deformed-discocyte (DD), Membrane internalization (MI) showing pesticide toxicity. X4,000

b: Deformed-discocyte (DD), Membrane internalization (MI), Spherocyte (SP) showing pesticide toxicity. X2,200

c: Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI) showing pesticide toxicity. X3,000

d: Deformed-discocyte (DD), Membrane internalization (MI) showing pesticide toxicity. X5,000
Fig. 15: RBC of albino mice showing abnormalities in Malathion pesticides (5 mg/kg body wt/day/30 days).

a: Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI), Spherocyte (SP), Stomatocyte (ST) showing pesticide toxicity. X2,700

b: Deformed-discocyte (DD), Echinocyte (E), Membrane internalization (MI), Spherocyte (SP) showing pesticide toxicity. X3,500

c: Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI) showing pesticide toxicity. X3,700

d: Deformed-discocyte (DD), Membrane internalization (MI), Echinocyte (E) showing pesticide toxicity. X4,000
Fig. 16: RBC of albino mice showing abnormalities in Malathion pesticides (10 mg/kg body wt/day/30 days).

a: Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI) showing pesticide toxicity. X2,700
b: Deformed-discocyte (DD), Membrane internalization (MI), Spherocyte (SP) showing pesticide toxicity. X3,500
c: Echinocyte (E), Deformed-discocyte (DD), Spherocyte (SP) showing pesticide toxicity. X3,700
d: Deformed-discocyte (DD), Membrane internalization (MI), Echinocyte (E) showing pesticide toxicity. X4,000
Fig. 17: RBC of albino mice showing abnormalities in Malathion pesticides (PSVT/kg body wt/day/30 days).

a : Deformed-discocyte (DD), Membrane internalization (MI) showing pesticide toxicity. X4,000
b : Deformed-discocyte (DD), Membrane internalization (MI), Spherocyte (SP) showing pesticide toxicity. X3,000
c : Deformed-discocyte (DD), Membrane internalization (MI), Stomatocyte (ST) showing pesticide toxicity. X2,700
d : Deformed-discocyte (DD) showing pesticide toxicity. X6,500
Fig. 18: RBC of albino mice showing abnormalities in Cypermethrin pesticides (5 mg/kg body wt/day/30 days).

a: Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI) showing pesticide toxicity. X6,000

b: Echinocyte (E), Deformed-discocyte (DD), Spherocyte (SP) showing pesticide toxicity. X8,000

c: Deformed-discocyte (DD), Spherocyte (SP) showing pesticide toxicity. X3,700

d: Deformed-discocyte (DD) showing pesticide toxicity. X2,700
Fig. 19: RBC of albino mice showing abnormalities in Cypermethrin pesticides (10 mg/kg body wt/day/30 days).

a : Echinocyte (E), Deformed-discocyte (DD), Membrane internalization (MI) showing pesticide toxicity. X3,500

b : Echinocyte (E), Deformed-discocyte (DD), Spherocyte (SP) showing pesticide toxicity. X4,300

c : Deformed-discocyte (DD), Spherocyte (SP) showing pesticide toxicity. X3,300

d : Deformed-discocyte (DD) showing pesticide toxicity. X3,000
Fig. 20: RBC of albino mice showing abnormalities in Cypermethrin pesticides (PSVT/kg body wt/day/30 days).

a: Echinocyte (E), Membrane internalization (MI), Spherocyte (SP) showing pesticide toxicity. X3,000
b: Deformed-discocyte (DD), Spherocyte (SP), Membrane internalization (MI) showing pesticide toxicity. X3,000
c: Deformed-discocyte (DD), Membrane internalization (MI), Stomatocyte (ST) showing pesticide toxicity. X4,500
d: Deformed-discocyte (DD), Echinocyte (E), Tear Drop (TD) showing pesticide toxicity. X1,700
Fig. 21: RBC of albino mice showing abnormalities in Fenvalerate pesticides (5 mg/kg body wt/day/30 days).

a: Echinocyte (E), Membrane internalization (MI), Deformed-discocyte (DD), Reticulocyte (R) showing pesticide toxicity. X3,700

b: Deformed-discocyte (DD), Spherocyte (SP), Reticulocyte (R) showing pesticide toxicity. X2,200

c: Deformed-discocyte (DD) showing pesticide toxicity. X3,700

d: Deformed-discocyte (DD), Membrane internalization (MI), Spherocyte (SP), Stomatocyte (ST) showing pesticide toxicity. X2,000
Fig. 22: RBC of albino mice showing abnormalities in Fenvalerate pesticides (10 mg/kg body wt/day/30 days).

a : Echinocyte (E), Membrane internalization (MI), Deformed-discocyte (DD), Spherocyte (SP), Spindle shaped (S) showing pesticide toxicity. X2,000

b : Echinocyte (E), Membrane internalization (MI), Deformed-discocyte (DD), Spherocyte (SP) showing pesticide toxicity. X1,500

c : Deformed-discocyte (DD), Spindle shaped (S), Stomatocyte (ST) showing pesticide toxicity. X6,000

d : Echinocyte (E), Membrane internalization (MI), Deformed-discocyte (DD), Stomatocyte (ST), Spherocyte (SP) showing pesticide toxicity. X3,300
Fig. 23: RBC of albino mice showing abnormalities in Fenvalerate pesticides (PSVT/kg body wt/day/30 days).

a: Tear Drop (TD) showing pesticide toxicity. X7,500
b: Membrane internalization (MI), Deformed-discocyte (DD), Spherocyte (SP) showing pesticide toxicity. X3,000
c: Membrane internalization (MI), Deformed-discocyte (DD), Spherocyte (SP) showing pesticide toxicity. X4,300
d: Membrane internalization (MI), Deformed-discocyte (DD), Stomatocyte (ST), Spherocyte (SP) showing pesticide toxicity. X3,300
e: Membrane internalization (MI), Deformed-discocyte (DD), Spherocyte (SP) showing pesticide toxicity. X3,300
Fig. 39: RBC of albino mice showing no abnormalities in Control group

a : RBC in normal shape. X 3,700
b : RBC in normal shape. X 2,000
c : RBC in normal shape. X 5,000
d : RBC in normal shape. X 2,500
Chapter 5

5. B. TRANSMISSION ELECTRON MICROSCOPIC STUDIES ON THE HISTOLOGY OF LIVER AND KIDNEY IN MAMMALS

INTRODUCTION

Pesticides have made valuable contributions to human health by increasing food and fibre production and by reducing the occurrence of vector-borne diseases (Blindauer et al. 1999). Unfortunately, while the acute toxicity of most pesticides is well documented (Ecobichon et al. 1999) information on chronic human illness resulting from pesticide exposure is not that sound (Wilkinson, 1990). Doll and Peto, 1981 has reported that occupational exposure of pesticides lead to some cases of all human cancers. The public health effects of pesticides cannot be denied. However, the undesired effects of chemical pesticides have been recognized as a serious public health concern during the past decades (Giri et al. 2003).

According to a 1997 market estimate, approximately 5684 million pounds of pesticides active ingredients are applied annually throughout the world
The World Health Organisation (WHO, 1992) reported that roughly three million pesticide poisonings occur annually and result in 220,000 deaths worldwide. Many of these chemicals are mutagenic (Galloway et al., 1987; Garaj-Vrhovac, 2000) linked to the development of cancers (Leiss & Savitz, 1995) or may lead to developmental deficiencies (Arbuckel & Server, 1998).

Pesticides occupy a rather unique position among the many chemicals that men encounters daily, in that they are deliberately added to the environment for the purpose of killing or injuring some form life. Ideally their injurious action would be highly specific for undesirable target organisms and non injurious to desirable, non-target organisms. In fact, however, most of the chemicals that are used as pesticides are not highly selective but are generally toxic to many non-target species, including men, and other desirable forms of life that co inhabit the environment. Therefore, lacking highly selective pesticidal action, the application of pesticide must often be predicated on selecting quantities and manners of usage that will minimize the possibility of exposure of non-target organisms to injurious quantities of these useful chemicals.

The benefits that the pesticides have brought to the society in terms of increased agricultural production and decrease in vector borne diseases cannot be denied. However, this had led to a tremendous increases and indiscriminate
uses of pesticides in agriculture, home and industry during the past 50 years. This might pose a serious threat to the genetic material of not only human beings, but also the surrounding flora and fauna. The importance to detect these chemicals in our environment has been recognised since a long time (WHO Scientific Group, 1971; Committee 17, 1975).

**REVIEW OF LITERATURE**

Toxicological evaluations of the hazard of handling and use of pesticides have for many years focused primarily on preventing injury to man, and common laboratory animals have severed as the experimental models for man’s biochemical, physiologic, and pathologic responses to these chemicals. Problems of species differences in susceptibility have always left some doubt concerning assignment of safe dosages for man on the basis of studies on common laboratory animals, but this approach appears to have been reasonably successful in protecting the general population in that there has not emerged any clear association between increasing use of pesticides and incidence of chronic diseases. However, occupational exposures have resulted in chronic or persistent neurologic disease status in the case of a few compounds.

Acute poisoning by pesticides also occur. They are usually the result of occupational exposures or of careless use, misuse, or mishandling the pesticides. The mortality rate attributed to poisoning by pesticides has been
estimated at 0.65 per million population in the United States, but it has also
been estimated that there are 100 nonfatal poisoning cases.

The liver plays an astonishing array of vital functions in the
maintenance and performance of the body. Some of these major functions
include carbohydrate, protein, and fat metabolism, detoxification and secretion
of bile. Therefore, the maintenance of a healthy liver is vital to overall healthy
and well being (Sukumaran et al., 2008).

There is now overwhelming evidence that some of these pesticides pose
potential risk to humans and other life form and unwanted side effects to the
environment. No segment of the population is completely protected against
exposure to pesticides and the potential serious health effects, though a
disproportionate burden is shouldered by the people of developing countries
and by high risk groups in each country. The world-wide deaths and chronic
illnesses due to pesticide poisoning is about one million per year.

The high risk groups exposed to pesticides include the production
workers, formulators, sprayers, mixers, loaders and agricultural farm workers.
During manufacture and formulation, the possibility of hazards may be more
because the processes involved are not risk free (Hurley et al., 1998). In
industrial setting, the workers are at increased risk since they handle various
toxic chemicals including pesticides, raw materials, toxic solvents and contain
environmental chemicals including pesticides termed as endocrine disruptors
are known to elicit their adverse effects by mimicking or antagonising natural hormones in the body and it has been postulated that their long-term, low-dose exposure are increasingly linked to human health effects such as immune suppression, hormone disruption, diminished intelligence, reproductive abnormalities and cancer (Crisp et al., 1998; Brouwer et al., 1999).

Observations confined to health surveillance in male formulators engaged in production of dust and liquid formulations of various pesticides (Malathion, methyl parathion, DDT and Lindane) in industrial setting of the unorganised sector revealed a high occurrence of generalized symptoms (headache, nausea, vomiting, fatigue, irritation of skin and eyes) besides psychological, neurological, cardio-respiratory and gastrointestinal symptoms coupled with low plasma cholinesterase activity (Gupta et al., 1984).

These suggest that pesticides affect immune system as well as detoxification tissues. Thus, exposure to these compounds via food may lead to the decline in immune activities of human body, resulting in diseases (Nishimoto et al., 2009).

The widespread environmental pollution caused by the chemical substances such as pesticides is a serious problem for creatures including human (McClure et al., 2001; McKinlay et al., 2008; Boobis et al., 2008), various chemical substances entering animal bodies are carried to the organs responsible for detoxification, such as liver and kidney, and excreted
(Nishimoto et al., 2009). On the other hand, some hydrophobic substances among these chemicals are accumulated in our body (Somogyi and Beck, 1993).

In some previous reports, influence of these pesticides on reproductive system has been studied (Welchons et al., 1999; Bretveld et al., 2007). Acetylcholin esterase is an enzyme that is essential for the normal functioning of the central and peripheral nervous system (Hand, 1993; Fribroulet et al., 1990). The transmitter substance is responsible for the transmission of signals from the terminals of motor neurons to the muscles. The organophosphate chemicals malathion affect the nerve cells (Mahananda et al., 2008). Pesticides effect on protein metabolism (Palanichamy et al., 1989). Toxic effect of cypermethrin and fenvalerate on carbohydrate content body wall muscle, kidney and liver have been observed (Ramu et al., 2008). The cypermethrin and fenvalerate is more toxic which result severe damage found in the tissue and cell (Ramu et al., 2008). Malathion is a widely accepted organophosphate insecticide that kills both vertebrate and invertebrates by inhibiting ChE with consequent disruption of nervous activity (Brien, 1967; Verma et al., 1981). The degree of ChE inhibition experience by an individual organism is a function of exposure of pesticides and body burden of residue (Busby et al., 1981).

Peoples are exposed to pesticides not only in pesticides spraying areas but also through the use of lawn and garden products and household insect repellents and anti-fungals. Maximum neurological problems occur only due to
exposures to environmental toxin, chemicals and pesticides. According to an estimate of the National Academy of Science, USA, the organophosphate, pesticides used in the US with sixty million pounds applied to agricultural and land, seventeen million pound used in residential and commercial applications annually, and exposure to these pesticides are linked to hyperactivity, behaviour disorders, leaving disabilities, developmental delays and motor dysfunction (Carol and Karins, 2008).

These insecticides, which are very persistent in soil are highly toxic to many arthropods and were being used widely since 25 years after the Second World War. They include compounds such as DDT, Benzene hexachloride, chrordane, heptachlor, toxaphene, methoxychlor, aldrin, dieldrin and Endosulfan, which are relatively non-soluble, have a low volatility and are lipophilic (Gupta, 1999).

These insecticides have carbon, hydrogen & chlorine as their basic molecular constituents but oxygen & sulphur are also present in some insecticides belonging to this group. They are also known as chlorinated organic or chlorinated hydrocarbon or chlorinated insecticides (Risebeough, 1986; Gupta, 1999).

Endosulfan is a neurotoxic organochlorine insecticide of the cyclodiene family of pesticides. It is highly toxic and is an endocrine disruptor. It is banned in several countries including Germany, Norway and the Philippines.
Endosulfan is also a xeno-estrogen, and it can act as an endocrine disruptor, causing reproductive and developmental damage in both animals and humans (Gupta, 1999). Endosulfan is one of the more toxic pesticides available in the market today, responsible for many fatal pesticide poisoning incidents around the world. It is a non-systemic contact and stomach poison. It is metabolized to corresponding sulphate in plants & mammals (Gupta, 1999).

The effect of the Endosulphan on mice kidney was investigated at ultrastructural level by Gupta et al (1999). The presence of mitochondrial degeneration in cytoplasm cells were a striking feature. Thus, this degeneration in kidney may be thought that oxidative stress may play a role to the mediator in changing configuration of cell membrane and seem to account for the morphologic alteration of kidney. Endosulphan, a polycyclic chlorinated hydrocarbon of cyclodien group is an organochlorine insecticide. It is widely used for agricultural insecticides. Hence, Endosulphan is important for environmental contamination. In addition, Endosulphan is used in plants such as corn, vegetable, cotton and tobacco (Gupta, 1999). Thus, the pesticide may enter into human and animal systems directly or via environmental contamination. The primary effect of Endosulphan is neurotoxicity after long term inhalation oral exposure. Experimental studies have shown that Endosulphan may cause inhibition of microsomal enzymes with regard to acute and subacute toxicity in liver, inhibition of enzymes of androgen
biosynthesis in testis and inhibition of mixed function oxidases in kidney (Kellogg and Fridovich, 1975; Inbaraj and Harder, 1983; Narayan et al., 1985; Singh and Pandey, 1989, 1990). Furthermore, marked hyperplasia and hypertrophy of tubuler cells in kidney are seen as a result of the effect of chronic exposure some pesticides such as malathion. Besides, an increase in excretion of potassium was significant (Bosco et al., 1997).

Organophosphate insecticides were first developed as nerve gases during the Second World War. They include parathion, diazion, trichlorfon, phorate, carbophenothion, disulfoton, dimethoate, fenthion, thionazin, menazon, dyfonate and chlofenvinphos. Although these chemical are much less persistent than the organochlorines many of them have much higher mammalian toxicities and potential to kill birds and other wildlife (Baird, 1995).

Those insecticides, having phosphorous atoms comes under the category of organophosphates. These insecticides can also be named as phosphate insecticides or organic phosphates etc. Organophosphate compounds are the combinations of different alcohols and different phosphorus acids. Thus, they are the esters of phosphorus (Gupta, 1999).

Absorption on organophosphate insecticides is taken place through skin, respiratory, gastrointestinal and circulatory system. The symptoms of organophosphate poisoning are mutagenic effects, nicotinic effects, central
nervous system effects and blood porphyrin uria. The organophosphates inhibit the cholinesterase enzyme of the nervous system of both vertebrates & insects.

Chlorpyriphos is one of the 100 organophosphate (OP) insecticides available in the market today. It is used to kill insect pests by disrupting their nervous system. Chlorpyriphos has an advantage over other products in that it is effective against a wide range of plant-eating insect pests. Chlorpyriphos poses a risk of serious damage to eyes, and is irritating to skin. Poisoning via the skin can easily be misdiagnosed suggesting some cases of occupational exposure in humans (Gupta, 1999). The WHO recommended that it can be rapidly detoxified in the animal body. It is non-systemic contact insecticides (Gupta, 1999). Chlorpyriphos, a neurotoxic organophosphate insecticide found to damage heart & liver cells. (Durban, 2003). Parental exposure to pesticides may contribute to childhood cancer risk. Through the study of pesticide applicators in Iowa & North Carolina, they find out childhood cancer risk and association with parental pesticide application (Flower et al. 1997).

Chlorpyriphos is a neuro-toxicant, the brain cell number (DNA concentrations and contents) cell size (protein & DNA concentrations and contents), cell size (Protein & DNA ratio), neurotic projections (membrane/total protein) were get effected by the exposure of organophosphate insecticides, chlorpyriphos. Reported by Rhodes, et al. (2010) environmental exposures
during the childhood asthma risk found due to large amounts of pesticides, pesticide usage in the agricultural field.

Malathion is one of the safest organophosphorous compounds now marketed in the form of emulsion concentrate. Malathion is a persistent general purpose insecticide particularly suitable for household, homegardens, vegetables and fruit pest control. It is highly toxic to mites and a number of insect species, while being generally non-phytotoxic. It has relatively low toxicity to higher animals (Gupta, 1999).

Malathion is commonly used insecticide, and has been employed in major eradication programmes in metropolis (CDHS, 1991). The malathion is reported to produce chromosomal aberrations and micronucleus in plants (Lindsley and Zimm 1992). Other studies on chromosome aberration and micronucleus in mammalian cells (Giri et al. 2002, Flessel et al. 1993) sister chromatid exchanges (Galloway et al. 1987, Flessel et al. 1993) and specific mutations in human T-lymphocytes (Pluth et al. 1996) indicate a potential mutagenic activity for malathion.

Thus, like the other organophosphorous pesticides, malathion primarily acts through inhibition of acetyl-cholinesterases (Perry et al. 1998), it may be cytotoxic at higher doses. Organophosphorus pesticides are reported to be relatively non-persistent and rapidly metabolized and eliminated from the body (Perry et al. 1998). The daily lower intake of the chemical may be quickly
detoxified by the host’s detoxification mechanism and eliminated from the body. Speit et al (2000) suggested that highly reactive compounds with a clear genotoxic potential may not induce a genotoxic effect in the target tissue at low concentrations. Because, either the mutagen and their reactive metabolites are metabolically inactivated, or they do not reach the DNA due to effective clearance processes at the cell surface or early reaction with non-DNA targets (Speit et al, 2000). Further, the repeated lower doses may lead to an adaptive response that enhances the resistance of the cells and gives additional capabilities to repair. The existence of an adaptive response has already been reported for other mutagens in other organisms (Moustacchi 2000, Cicchetti et al. 1999, Rigaud and Moustacchi 1996).

Organophosphorus compounds have been reported to have alkylating properties (Garrett et al. 1990, Wild 1975) and the methyl esters have a higher alkylating potential than the ethyl esters (Garrett et al. 1990). Alkylating agents have been reported to cause DNA damage (Ferguson and Denny 1995). The phosphorus in the organophosphorus pesticides may act as a good substrate for nucleophilic attack and may cause the phosphorylation of DNA resulting in DNA damage (Bhuiyan and Jena 1993). Most genotoxic carcinogens are electrophilic by themselves or may be activated to electrophilic intermediates that bind to critical macromolecules (Searle 1984). The mutagenic activity of malathion may be due to the existence of electrophilic sites in the parent
molecule or its metabolic intermediates which are capable of binding to nucleophilic sites in DNA. In malathion, there are two potential electrophilic sites – the alkyl group(s) and the phosphoryl group (Blasiak et al. 1999). Some organophosphorus compounds are reported to have the ability to bind to DNA (Wauchope et al. 1992) and cause mutations (Rehana et al. 1996, Valkova et al. 1993, Kappas et al. 1990).

Malaoxon formed by oxidation of malathion, and isomalathion formed by isomerisation of malathion (Berkman et al. 1993). Malaoxon and isomalathion have been reported to be mutagenic (Blasiak et al. 1999, Flessel et al. 1993). Malathion was implicated in the epidemic malathion poisoning of 2800 Pakistani spray men during a malaria control programme (Baker et al., 1978). The large scale use of malathion in various eradication programmes has raised concern over its potential to cause genetic damage (Flessel et al., 1993). Most of the studies on the mutagenic effects of malathion are supported from in vitro studies (Galloway et al., 1987; Herath et al., 1989; Blasiak et al., 1999).

Synthetic pyrethroids are synthesized derivatives of naturally occurring pyrethrins. The insecticidal properties of pyrethrins are derived from ketoalcoholic esters of chrysanthemic and pyrethroic acids. These acids are strongly lipophilic and rapidly penetrate many insects and paralyze their nervous system (Reigart et al. 1999). Both pyrethrins and synthetic pyrethroids are sold as commercial pesticides used to control pest insects in agriculture,
homes, communities, restaurants, hospitals, schools, and as a topical head lice treatment (Mueller & Doria, 1990). Low toxicity is attributed to two factors: limited absorption of some pyrethroids, and rapid biodegradation by mammalian liver enzymes (ester hydrolysis and oxidation). Insects, without this liver function, exhibit greater susceptibility to the chemicals (Reigart et al., 1999).

WHO (1990) reported that synthetic pyrethroids are neuropoisons acting on the axons in the peripheral and central nervous systems by interacting with sodium channels in mammals and/or insects. Exposure to pyrethroids has resulted in contact dermatitis and asthma-like reactions. The symptoms of acute toxicity due to inhalation include sneezing, nasal stuffiness, headache, nausea, incoordination, tremors, convulsions, facial flushing and swelling, and burning and itching sensations. The most severe poisonings have been reported in infants, who are not able to efficiently break down pyrethroids (ETN, Pyrethroids, 1996).

Many pyrethroids have also been linked to disruption of the endocrine system, which can adversely affect reproduction and sexual development, interfere with the immune system and increase chances of breast cancer. Pyrethroids contain human-made, or xenoestrogens, which can increase the amount of estrogen in the body (Garey et al., 1998). Synthetic pyrethroids effects on persons engaged in packaging fenvalerate in China documented
burning sensations, lightness or numbness on the face. Sniffles, and Sneezes.
Other symptoms included abnormal facial sensations, dizziness, fatigue & skin
rashes (He et al, 1989).

Exposure to pyrethroids and organophosphates has also been shown to
increase the inhibition by the organophosphates of cholinesterase, an enzyme in
the nervous system (Gaughan et al., 1980; Abiola, 1988). Acute and subacute
studies have shown that the main effects of pyrethroids are neurotoxicity at
high doses and liver hypertrophy (enlargement of the liver) reported by
pyrethroids can be mildly to severely irritating to the skin and eyes [Uclaf,
(1982); Litchfield, (1985); FMC Corporatio (1988); FMC Corporation (1989)].
Some pyrethroids also cause a sensitization of facial skin which has been
observed to be reversible (Litchfield, 1985). The dermal (skin) toxicity of some
pyrethroid formulations is greater than that of the technical grade. Adverse skin
effects were not measured in tests on nonhuman animals (Litchfield, 1985;
Hayes and Wayland, 1982). Other chronic effects are reduction in the growth
rate of test animals, liver enlargement, and an increase in the activity of some
enzymes in the liver; these changes are not fully reversible (Desi, 1986). Chronic
exposure studies have also measured effects on the adrenals, spleen, pituitary
and testes (Litchfield, 1985). Other possible effects include suppression of the
immune system and damage to the nervous system [Desi, (1986). Chambers
Effects on reproduction have been observed with several pyrethroids and pyrethrins. [Litchfield, (1985); International Registry of Potentially Toxic Chemicals, United Nations Environment Programme, (1990); National Coalition Against the Misuse of Pesticides, 1987; Ruzo et al., 1977]. In a number of separate mutagenicity studies (studies of the ability to cause genetic damage), cypermethrin has shown some mutagenic effects (Litchfield, 1985; Catinot, et al. 1989). Carcinogenicity studies of permethrin, resmethrin, fenvalerate and deltamethrin have shown increases in various kinds of cancers (Litchfield 1985; Desi, et al., 1986; Cabral and Galendo,1990). Only permethrin has been determined to be a potential or weak carcinogen by the U.S. Environmental Protection Agency (EPA, 1979). Carcinogenicity studies have also been done on phenoethrin, allethrin, and cypermethrin; none were carcinogenic (Litchfield, 1985; Cabral, 1990). A study of synthetic pyrethroids' effects on persons engaged in packaging fenvalerate and deltamethrin in China documented burning sensations, tightness or numbness on the face, sniffles, and sneezes. (He, et al., 1989).

Overexposure of synthetic pyrethroids effects on human skin resulting in paresthesias can occur (Flannigan,1985). Systemic intoxication is uncommon in humans, as the dermal absorption of these chemicals appears to be minimal
(Woollen et al. 1992; Williams et al. 2003). Most cases of systemic poisoning and central nervous system effects from synthetic pyrethroids have been reported in association with occupational overexposure and from intentional ingestion (Le et al. 1980; He et al. 1989).

Individuals at higher risk of continuous exposure to synthetic pyrethroids include workers involved in the application of these compounds with direct contact with synthetic pyrethroids include transferring, mixing, and diluting concentrated formulations (He et al. 1989; Tucker 1983). Continuous exposure can also occur from moving through vapour or mist at or around the time of application.

In metastatic or primary liver cancer, the liver becomes infiltrated with deposits of cancer cells that can grow rapidly (Castell et al. 1989). Electron microscopic morphometry has demonstrated a rapid decrease in the fractional volume of autophagic vacuoles (AV) in hepatocytes and decrease in glycogen of adult male rats. This indicate that autophagy plays an important role in intracellular turnover (Feifer, 1978).

Fenvalerate is an insecticide of moderate mammalian toxicity. Fenvalerate has applications against a wide range of pests. Residue levels are minimized by low application rates (Gupta, 1999). Symptoms of poisoning through direct contact include dizziness, burning and itching (which is worsened by sweating and washing), blurred vision, tightness in the chest, and
convulsions. When ingested by laboratory animals, symptoms of poisoning include muscle in coordination, tremors, convulsions, nerve damage, and weight loss. Fenvalerate is a strong eye irritant and a suspected endocrine disruptor (Kolmodin, 1982).

Cypermethrin, one of a handful of light stable synthetic pyrethroids, is registered to control a variety of household pests. It is also useful in agriculture to control pests on cotton, fruits and vegetable (Gupta, 1999). About 90% of the cypermethrin manufactured worldwide is used to combat pests feeding on cotton crops (WHO, 1990). After treatments in the home, cypermethrin persists for about three months (Wright et al., 1993). Symptoms of cypermethrin poisoning in humans include numbness, burning, loss of bladder control, vomiting, in coordination, seizures, coma and death (Go, 1999).

Cypermethrin is a very active synthetic pyrethroid insecticide and is used to control pests of a variety of crops. Giray et al. (2001) reported that cypermethrin exposure of rates resulted in free radical mediated tissue damage and reduced the total glutathione (GSH) level by 20%. Hemming et al. (1993) in a study using partially hepatectomised male Sprague-Dawley rats, reported that cypermethrin as a single agent enhanced the development of N-nitrosodiethulamine (NDEA)-initiated GGT-positive foci in the liver at non-hepatotoxic doses. They suggested that cypermethrin could act as a tumor promoter.
Cypermethrin has been reported to induce gene mutations in male germ cells of Drosophila (Batiste-Alentorn et al., 1986) and genotoxicity and sperm abnormality in mice (Bhuiya and Pati, 1988).

Synthetic pyrethroids account for more than 30% of insecticides used worldwide in agricultural, domestic and veterinary applications and have a high potential for human exposure (Eisler, 1992; Perry et al., 1998). Cypermethrin is reported to cause free radical-mediated tissue damage and reduce total GSH in rats (Giray et al., 2001).

To indicative liver cell changes (lipofucinosis) and adaptive reactions (hypentrophy of the endoplasmic reticulun) lesions of the hepatocytes usually reflective of primary toxic reactions (single cell necroses, toxic cell swelling, fatty infiltration were observed. Acute & sub acute studies have been shown that the main effects of synthetic pyrethroids are neurotoxicity at high doses and liver hypentrophy (enlargement of the liver) Litchfield, in 1985 & Litchfield in 1983. Other chronic effects are reduction in the growth rate of test animals, liver enlargement and an increase in the activity of some enzyme in the liver (NRCC, 1986 & Mueller et al., 1990). In number of mutagenicity studies shows the ability to cause genetic damage by cypermethrin & fenvalerate reported by Litchfield (1985); IRPTC (1990); Cationot et al. (1989).

The liver is the largest organ in the human body. The normal liver is smooth, with no irregularities. The most common disease associated with a
palpable & enlarged liver include metastatic cancer, lymphoma, longstive heart failure etc. In metastatic or primary liver cancer, the liver becomes infiltrated with deposits of cancer cells that can grow rapidly. Liver cell enlargement can occur in lymphoma. Hepatitis or other chemical cause of fatty liver lead to linear enlargement of liver by hepatocytes enlargement (Douglas, 1990).

The main function of the kidney is the removal of toxic waste products from the blood. Chief among these waste products are urea and uric acid. If too many of these waste products are allowed to accumulate in the blood stream, this will result in life threatening illnesses. Fortunately, these two deadly substances are easily expelled from the body by the kidneys through the process of excretion.

Nucleus is one of the most prominent cellular organelles, and its shape and size are important in cellular function (Webster, et al., 2009). Abnormal nucleus shape was reported to reflect mitotic instability which is bound to affect the body growth adversely (Gisselsson, et al., 2001). The role of abnormal nuclear shape in impaired mechano–transduction has been reported. Lammerding et al., 2005. The relation between structure and body function in vertebrate has been reported by Nikolva, et al., 2004. The nuclear abnormalities (fragmented apoptotic cells after exposure to pesticides in kidney by Barsiene et al. (2006). The haematological micronucleus is regarded as an indicator of the
clastogenic effect of chemicals and acute cytogenic damage (Gerhand et al. 1985).

The nuclear envelope is composed of two membranes joined at regular intervals to form circular openings called nuclear pores. DNA and proteins associate to form a network of threads called chromatin. Inside the nucleus is a filamentous region called the nucleolus. This serves as a site where the RNA and protein components of ribosomes are assembled. It controls all vital metabolic activities of the cell and it also contains genetic material. Nucleus plays an important role in cell replication. Variation in size and shape reflects abnormal cell division and maturation in nucleus (Zink et al., 2004). The changes in nuclear shape lead to changes in chromosome organization, which in turn can affect gene expression (He et al., 2008). The abnormalities in nuclear shape is the rigidity of the nucleus and in chromatin recognition and thereby affect gene expression (Capell et al., 2005; Glynn and Glover, 2005; Mallampalli et al., 2005; Toth et al., 2005). The nuclear size is important for cell function. Disturbance of this ratio is associated with certain types of cancers (Slater et al., 2005; Zink et al., 2004). The cell-cycle progression depends on nuclear size (Roca-Cusachs et al., 2008; Yen and Pardee, 1979) and the cell cycle (Futcher, 1996). A strong correlation between nuclear size, RNA transcription levels and cell size has been found (e.g. Sato et al., 1994; Schmidt and Schibler, 1995). The volume of the nucleus might be important for maintaining nuclear
compartments, such as the nucleolus, and the activity of enzymes such as DNA polymerase, which are sensitive to macromolecular crowding (Hancock, 2004; Miyoshi and Sugimoto, 2008; Sasaki et al., 2006).

Variation in size & shape reflects abnormal cell division and maturation. Significant variation in nuclear size (Anisonucleosis) reflecting abnormal cell division and maturation & abnormal shape are also shows the morphological features of cancer cells (Malignant cells). Abnormal nuclear shape in solid Tumours reflects mitotic instability by David Glisselsson et al. in 2001. Treatment of rats with low doses of pesticides is associated with a number of phenomena, including nuclear enlargement and altered nucleocytoplasmic compartmentation, which potentially reflect initiatory changes. Carcinogens induce nuclear enlargement, which is generally associated with increases in DNA content.

Treatment of rat with very low doses of pesticides induced by nuclear enlargement and altered nucleocytoplasmic compartmentation, which potentially reflects initiatory changes (Clawson et al. 1992). Since a number of investigations have indicated that changes in nuclear shape may relate to development of hepatocellular carcinomas in liver. The process by which chemical carcinogens produce cancer is complex, encompassing initiation, promotion and in transformation to a malignant phenotype (Berenblum et al. 1954, Boutwell 1974 and Goldsworthy 1989).
This nuclear enlargement is a general feature carcinogen action. It is of considerable interest here because it occurred in the absence of histological evidence of toxicity or nuclear replication (Clawson et al. 1984, Olason et al. 1976). The nuclear enlargement in the liver cells indicates hepato-cellular carcinoma in liver (Gary et al., 1992).

An initiatory carcinogen action is the permanent elevation of nucleoside Niphosphatase activity (Clawson et al. 1984, Olason et al. 1976), an enzyme which is regulate nucleoyto plasmic RNA transport (Anutten et al. 1979, Baglla et al. 1983, Clawson et al. 1984, Purrello et al. 1983, Punnello et al. 1982).

An increase in the size of hepatocyte nuclei is the common signs of toxic effects, e.g., cell death, fat vacuoles or loss of glycogen. The nuclear enlargement can provide a valuable parameter for the evaluation of carcinogen effects (Enzmann et al. 2010).

Mitochondria generate energy for all vital activities of the cell. Mitochondria perform generally four types of function. They are cell respiration, ATP transport, Lipid synthesis and Elongation of fatty acids. They produce energy rich ATP molecules from food, oxygen and ADP. The process involves a number of complex biochemical reactions. Mitochondria perform oxidation, dehydrogenation and oxidative phosphorylation.

Consumption of pesticides at least six months to 8 months entails biochemical and histological alternation in liver mitochondria of albino mice.
Decreased oxidation roles of certain mitochondria substance, decrease in the lent of intra & extra mitochondria thamine A phosphate & increased aclivity of certain hydrolyzing enzymes have been observed Electro micro scope studies reveal a high percentage of markedly malformed mitochondria reported by Kiessling et.al, 2009.

The above study on the effect of pesticides on liver of albino mice also revealed mitochondria with abnormal vacuolization inside the organelle. Mitochondrial cristae also showed breakage, cristae were very light, mitochondrial wall ruptures (outer wall) and swollen of mitochondria are found. Extensive distortions of inner membrane of Mitochondria were also observed in the microscopic study. These structural changes were associated with pronounced increases In the Specific activities of the mitochondria membrane associated enzymes.

A detailed study correlating changes in mitochondrial optical density, ultrastructure of mitochondria (swelling) in liver & heart of rat liver were reported (Stoner & Sirak, 1969).

The cytological changes induced in rat liver cells by using Azodye observed carcinogenic effect of the hepatic cells (Lafotraine, 1964).

Autophagic cell death is morphologically characterized by an accumulation of autophagic vacuoles. A huge quantity autophagic vacuoles the damaged nucleus & Mitochondria. Evidence implicating Mitochondria as
playing a crucial role in both necrotic and apoptotic cell death is rapidly accumulating. Mitochondria one essential in controlling specific apoptosis pathways (Green & Reed 1998). Mitochondria damaged is very relevant in many of the cell infected either in vivo or in vitro (Martin 1996; Martin & Anderson, 1997 and Martin and Anderson, 1999). Mitochondria primarily serve as the major source of cellular chemical energy (Lodish et al., 2000).

Mitochondrial swelling, disruption of cristae and marked cavitation of space; in some sections of a glycogen accumulation was observed in ultrastructure of guinea pig hearts in the effect of sodium selenite (Dini et al., 2004). Mitochondrial swelling and membrane potential loss occurred prior to cell death in plant (Panda et al., 2008). An ultrastructural study of Tobacco cells increasing levels of mitochondrial distortion, swelling and dissociation was observed (Cole et al., 1988). Active changes in volume and shape of mitochondria may be caused by chemical, osmotic and mechanochemical changes. Mitochondria are one of the most sensitive indicators of injury to the cell.

Vacuolazation observed in the mitochondrial matrix is significant because of the fact the auto phagic cell death is morphologically characterized by an accumulation of vacuoles (Gonzalerg et al., 2005). Starvation can induce mitochondrial autophagy, which is required for the synthesis of proteins essential to survival under starvation condition. (Cavallini et al., 2007).
Mitochondrial outer & inner membrane fusion & cristae maintenance has been reported (Meeusen et al., 2006). Autophagic vanuolization found in nutrient depleted cells (KIESS ling et al., 1964) in animal cells hypoxiz & anoxia cause elongation & disc-shaped mitochondria (Bereiter & Voth, 1983), but also respiratory inhibitors and uncouplers provoke disc-shaped mitochondria (Bereiter & Voth 1983; Markova et al., 1990). The association between megamitochondria apoptosis in culture rat cells generating by pesticides induced large mitochondria.

The importance of mitochondrial function in determining not only aging & life span, but also anaesthetic sensitivity were observed (Kaysen et al., 2001). Mitochondria structural is mainly based on biochemical stimuli (Perkins & Frey 1999). The cytoplasm matrix is traversed by an inter-connecting network of membrane bound tubules or cavities. Cells which are actively engaged in protein synthesis contain highly developed endoplasmic reticulum. (Higashio et al., 2000; Matynia et al., 2002).

The endoplasmic reticulum is a dynamic organelle central to many essential functions. It is an important calcium store, which functions in cellular signal transduction casecades. It is also the site of entry secreted proteins into the sectary pathway. The endoplasmic reticulum is also the site where most cellular lipids are synthesized. It is continuous with the nuclear envelope which serves as a diffusion barrier to control entry into and out of the nucleus. In the
life cycle of cell, the endoplasmic reticulum is in a constant flux of membrane traffic (Powell et al. 2000).

Ultrastructural studies were conducted to evaluate the effects of pesticide on organelle structure in albino mice in liver and kidney cells. The five types of pesticide was administered subcutaneously to male in (Fowlen, 1987) different concentrations mg/kg was given Electron microscopy revealed swollen mitochondria and distortion of mitochondrial in membranes in liver & this studies demonstrate that the initial acute effects of pesticides in liver and kidney cells distortion of mitochondrial membranes. Both effects contribute to compromise of membrane associated enzymatic functions (Woods & Flower, 1987).

Ribosome are the site of active protein synthesis. Most of these proteins are transported into or across the membrane as they are synthesized, accumulating in completed form within either the membrane or the lumen of the endoplasmic reticulum. Not all proteins are synthesized on the rough endoplasmic reticulum, however, maximum protein synthesis occurs on ribosomes that are not attached to the endoplasmic reticulum but are instead forum free in the cytosol.
MATERIALS AND METHODS

The experiment was carried out to observe the effect of pesticides on the red blood cells of albino mice. Swiss albino mice originally obtained from Pasture Institute, Shillong, India. The animals were housed in polypropylene cages (10 animals per cage with Sawdust as the bedding material) at 25±5°C temperature on a 12h light/dark cycle. The animals were supplied with dry food pellets commercially available (Hindustan Liver Ltd., New Delhi) and water. Healthy male animals in the age group of 10-12 weeks and weighting 20-25 g were used as test animals.

Transmission Electron Microscope

Fixation was achieved by sacrifice an animal take out the liver washed in distilled water. Cut into a 1 mm cube size keep in 2.5 – 3% Kannovsky’s fixations for 4 hours in 4°C temperature for fixation purpose. After 4 hours the sample then washed with 0.1 M Sodium Cacodylate Buffer 3 changes of fifteen minutes each at 4°C. After primary fixation post fixation started with 1% osmium teraoxide in 0.1 M Sodium Cacodylate Buffer at 4°C for a hour : keep out the sample from osmium tetraoxide & add the 0.1M Sodium Cacodylate Buffer 3 charges of 15 minutes each at 4°C. After washing he sample with buffer dehydration process started with autone of different gradation 2 charges of 15 minutes each at 4°C. After completion of all dehydration process keep the
sample 100% autone for 30 minutes at room temp. After that clearing is carried out Propylene Oxide at room temperature for 15 minutes of 2 charges. Then Infiltration with Propylene Oxide : embedding Medium 3:1 for Overnight. Then next for 1:1 for 1 hour room temp. & 1:3 for 1 hour in vacuum. Lastly the sample place in pure embedding medium for 1 hour at 500C oven. The infiltration samples are transferred into embedding moulds at 500C in oven for overnight.

After completion of imbedding polymerization process started. The embedded blocks are kept at 500C in a oven for 12 to 24 hrs. the temp. is then raised to 600C and the embedded tissues are kept for 24 to 48 hrs to complete the process of polymerization. The blocks are taken for ultrathin sectioning 60 – 90 mm sections are cut with diamond knives under the ultramicrotime.

The ultrathin sections on the water are stretched by exposing them to chloroform. Coper grids are used for keep the section on the grid 3% polyrengl formal dehyde in ethlyex dichloride are used for coating the grid. The ultrathin section were stained with double staining method using uranyl acetate & than lead citrate for 10 – 15 min & washed with Sodium hydhroyide & than with doubled distilled water A staining helps in enhancing the contrast of biological samples. Dry the grid in inside the laminar aim flow. The sections are ready for observation in the Transmission Electron Microscope.
Chlorpyriphos (C₉H₁₁Cl₅NO₃PS)

O,O-diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate
Chloropyrifos 20% EC (Kaman) mfd. by Crystal Phosphate Ltd.

Malathion (C₁₀H₁₉O₆PS₂)

[S-1,2–bis(ethoxycarbonyl) ethyl O, O-dimethyl phosphorodithioate]
Malathion (Kunamala 50) 50% EC, Kundu Agrochemical Pvt. Ltd.

Endosulfan (C₉H₆Cl₆O₃S)

Endosulfan (Thiodan) 35%, Bayer Crop Science Ltd.
1,4,5,6,7,7-Hexachloro-8,9,10-trinorborn-5-en-2,3-ylenebismethylene) sulfite

Cypermethrin (C₂₂H₁₉Cl₂NO₃)

[Cyano (3-phenoxyphenyl) methyl – 3- (2, 2-dichloro-ethenyl), 2, 2-dimethylcyclopropanecanboxylate
Cypermethrin (K-HERO) 10%EC, Kundu Agrochemical Pvt. Ltd.

Fenvalerate (C₂₅H₂₂ClNO₃)

α-Cyano-3-phenoxybenzyl-(s)-2-(4-chlorophenyl)-3-methylbutyrate
Fenvalerate 20% EC, Rallis India Ltd., Agrochemicals Division

For the experiment five types of pesticides were selected from different chemical groups i.e., malathion, cypermethrin, endosulfan, chloropyrifos, fenvalerate for albino mice. Three different doses were selected for the
experiment with concentrations of 5mg/kg, 10 mg/kg and PSVT (pesticides sprayed vegetables treatment) and control groups received normal water. After 30 days and 60 days of above treatments, the blood taken from the albino mice and slides were prepared for transmission electron microscopic study.

RESULTS

OBSERVATIONS UNDER TRANSMISSION ELECTRON MICROSCOPE

Deformities in liver tissues

Organochlorides

Endosulfan treated subjects (5mg/kg body wt./30 days) showed ruptured mitochondrial membranes. Cristae appeared very light. Endoplasmic reticulum was irregular & very few structure with free ribosomes in the cytoplasm. The nucleus is also abnormal in shape (Fig. 1 & 2).

Endosulfan treated subjects (10mg/kg per body wt./30 days) showed abnormal shape of nucleus with dark mitochondria. The mitochondrial membrane showed rupturing here and there. Endoplasmic reticulum is long but discontinuous. Ribosomes are not attached with endoplasmic reticulum and are free in the cytoplasm (Fig. 3 & 4).

Endosulfan treated subjects (PSVT/kg body wt./30 days) nucleus showed mild effect but mitochondria get effected largely. The mitochondrial wall found ruptured. Cristae are very light. Endoplasmic reticulum showed
breakage and the ribosome are totally free in the cytoplasm. Very few structure of endoplasm reticulum is found. The mitochondrial structures are very dark (Fig. 5 & 6).

Endosulfan treated subjects (5mg/kg body wt./60 days) showed abnormal shape of nucleus. Outer and inner membrane of some portion ruptured. Mitochondrial inner and outer membrane also got ruptured. Some of the mitochondrial matrix got mixed with cytoplasm. Cristae looked very light. In Endoplasmic reticulum also, a number of light structures were seen. Ribosomes appeared set free in the cytoplasm. The mitochondrial structure were found overlapping with each other (Fig. 7 & 8).

Endosulfan treated subjects (10mg/kg body wt./60 days) showed abnormal shape of nucleus. The outer membrane ruptured in some portion of nucleus. Mitochondrial inner and both outer membrane ruptured. The mitochondrial matrix come out from the mitochondria and mix in cytoplasm. The endoplasmic reticulum structure found long but discontinuous. Cristae are very light. Ribosomes are set free in the cytoplasm (Fig. 9 & 10).

Endosulfan treated subjects (PSVT/kg body wt./60 days) showed abnormal shape of nucleus. The mitochondrial membrane (both inner and outer) were seen to have ruptured. Cristae were less in number. Very few and light structure of endoplasmic reticulum were found. Maximum number of
mitochondrial matrix got intermingled with cytoplasm. Ribosomes appeared to have been set free in the cytoplasm (Fig. 11 & 12).

**Organophosphate**

Chlorpyriphos treated subjects (5 mg/ kg body wt./ 30 days) showed mitochondrial distortion of both in the inner and outer membrane. Cristae are very light. Endoplasmic reticulum appeared discontinuous. Ribosomes are totally free in the cytoplasm. Most of the mitochondrial membrane disappeared. The nucleus looks like an amoebic shape with nucleolus. Cell membrane appeared disappearing in one side (Fig. 13 & 14).

Chlorpyriphos treated subjects (10 mg/ kg body wt./ 30 days) showed amoebic shaped nucleus with finger like projection in both the nucleus. In mitochondria membrane distortion were observed. Endoplasmic reticulum were also found irregular. Ribosomes set free in the cytoplasm. The breakage of cristae were observed (Fig. 15 & 16).

Chlorpyriphos treated subjects (PSVT/ kg body wt./30 days) showed nuclear membrane totally disappeared in one side, mitochondria, that are adjacent to nucleus showed membrane distortion. Mitochondrial membrane showed rupturing. Very few endoplasmic reticulum structure were found. Ribosomes are totally free in the cytoplasm (Fig. 17 & 18).
Chlorpyriphos treated subjects (5mg/kg body wt./60 days) showed abnormal shape in nucleus. The mitochondrial membrane (both inner and outer) were ruptured. Different shape of mitochondria was found. One cristae was prominent in most of them but matrix got mingled with cytoplasm. Endoplasmic reticulum were very light. Ribosomes were totally free in the cytoplasm (Fig. 19 & 20).

Chlorpyriphos (10 mg/ kg body wt./60 days) treated subjects showed abnormal shape of nucleus with two nucleolus. The mitochondrial membrane both inner and outer were ruptured in most cases. The mitochondrial matrix mixed in cytoplasm. Cristae are very light. Endoplasmic reticulum breakage are also found. The ribosomes are free in the cytoplasm (Fig. 21 & 22).

Chlorpyriphos (PSVT /kg body wt./60 days) treated subjects showed mild effect in nucleus with different shape of mitochondria in the cytoplasm. The mitochondria were very dark in different shape. Vacuole were found inside the mitochondria. The cristae were very light. Endoplasmic reticulum were not found in the cytoplasm. Ribosomes set free in the cytoplasm (Fig. 23 & 24).

Malathion treated subjects (5mg/ kg body wt./30 days) revealed that the nucleus to be abnormal in shape with small finger like projections. The mitochondrial membrane, (both inner and outer) was ruptured. In most of the mitochondria cristae were very light. Endoplasmic reticulum are very few in
number. Ribosomes are totally free in the cytoplasm. Mitochondria are also found to be abnormal (Fig. 25 & 26).

In Malathion treated subjects, (10mg/kg body wt./30 days) both the inner and outer membrane of mitochondria were ruptured. Cristae were prominent. Very light structure of endoplasmic reticulum were found. Ribosomes were free in the cytoplasm. The structure of the nucleus was found to be abnormal in shape. The outer membrane of nucleus disappeared. The mitochondria got badly affected (Fig. 27 & 28).

Malathion treated subjects (PSVT/kg body wt./30 days) showed abnormal shape of nucleus. The outer membrane were very light. Mitochondria found to be abnormal in shape. The mitochondrial outer & inner membrane were ruptured. Endoplasmic reticulum structure were very light and few in number. Ribosomes are free in the cytoplasm. The outer and inner membrane are disappeared extremely. The cristae are also damaged (Fig. 29 & 30).

Malathion (5mg/kg body wt./60 days) treated subjects showed abnormal shape of nucleus. In some portion of nucleus outer membrane were ruptured. Mitochondrial membrane were damaged. Fewer number of cristae were found. Mitochondrial matrix got mixed with cytoplasm. In endoplasmic reticulum breakage in structures were found. Ribosomes were free in the cytoplasm (Fig. 31 & 32).
Malathion (10mg/kg body wt./60 days) treated subjects showed nuclear abnormality. The outer membrane of the nucleus disappear in some portion of the nucleus. Mitochondria get affected severely in malathion treated tissues. In mitochondria, both the membrane got ruptured. The mitochondrial matrix were seen mixed up with cytoplasm. Endoplasmic reticulum were found discontinuous. Cristae were found very light in some mitochondria and few were dark & prominent in others. Ribosomes were set free in the cytoplasm (Fig. 33 & 34).

Malathion (PSVT/kg body wt./60 days) treated subjects showed nucleus get effected for their abnormal shape with dark mitochondrial structure. Different shape of mitochondria were found, some were round, oval and some are elongated structure found. Maximum number of mitochondrial membrane showed rupturing. The cristae were very less number found. Very few structure of endoplasmic reticulum was found. Ribosomes were very few number found in the cytoplasm (Fig. 35 & 36).

**Synthetic Pyrethroids**

Cypermethrin treated subjects (5 mg/kg body wt./30 days) showed abnormal shape in nuclei. The nucleus looked like a triangular shape. The mitochondrial outer and inner membrane got ruptured due to the effect of cypermethrin on mitochondrial membrane. Cristae were very light. Endoplasmic structure were
found very irregular and breakage. Ribosomes were free in the cytoplasm and very few in number. The mitochondria were extremely damaged (Fig. 37 & 38).

Cypermethrin treated subjects (10mg/kg per body wt./30 days) showed abnormal shape in nucleus with mitochondria. The inner and outer membrane of mitochondria could not be identified separately, because it got extremely affected. Cristae also disappeared. Endoplasmic reticulum structure were very few in number and ribosomes were set free in the cytoplasm (Fig. 39 & 40).

Cypermethrin treated subjects (PSVT/kg per body wt./30 days) showed abnormal shape in nucleus with folded in one side. The outer and inner membrane also disappeared in nucleus. Mitochondrial inner and outer membrane disappeared in most of the mitochondria. Endoplasmic reticulum disappeared, but only structure could be seen. The ribosome appeared totally free in the cytoplasm. Abnormal mitochondrial shapes were found (Fig. 41 & 42).

Cypermethrin (5 mg/kg/body wt/60 days) treated subjects showed abnormal nuclear shape. Mitochondrial membrane,( both outer and inner) showed rupturing here and there. The matrix got mixed with cytoplasm. The endoplasmic reticulum was found to be very long with no ribosomal attachment. Endoplasmic breakages were also found in the cytoplasm. Ribosome appeared set free in the cytoplasm (Fig. 43 & 44).
Cypermethrin (10mg/kg body wt./60 days) treated tissues showed abnormal shape of nucleus. The mitochondrial damage by cypermethrin is very high. No mitochondrial membranes were found. The mitochondrial matrix got mixed up in the cytoplasm. Cristae are very less in number. Endoplasmic reticulum disappears totally. The matrix in the mitochondria is very light. Ribosomes were totally free in the cytoplasm (Fig. 45 & 46).

Cypermethrin treated tissues (PSVT/60 days) shows the nucleus to be affected badly. The outer membrane of nucleus ruptured. The mitochondrial inner and outer membrane also ruptured. The different shapes of mitochondria were found. The cristae were very light. Most of the cristae disappear. Light structures of endoplasmic reticulum were observed. Ribosomes are free in the cytoplasm (Fig. 47 & 48).

Fenvalerate treated subjects (5 mg/ kg body wt./ 30 days) showed abnormal shape of nucleus. Outer membrane was found to disappear. Different shape of mitochondria was found. Fenvalerate affected the shape of mitochondria very badly. The outer and inner membrane got ruptured. The cristae appeared very light. Endoplasmic reticulum was found to be discontinuous and breakage. Ribosomes were totally free in the cytoplasm (Fig. 49 & 50).

Fenvalerate treated subjects (10 mg/ kg body wt./30 days) showed abnormal nuclear shape. The mitochondrial membrane disappears. Both outer
and inner membrane ruptured. Cristae are very light. Endoplasmic reticulum is long with no ribosome attached. Ribosomes were free in the cytoplasm (Fig. 51 & 52).

Fenvalerate treated subjects (PSVT/kg body wt./30 days) showed abnormal nuclear shape with endoplasmic reticulum and mitochondria. The mitochondrial outer and inner membrane were ruptured. One of the most observable findings is the presence of different shape of dark shaded mitochondria. Cristae were very light. Endoplasmic reticulum was very few. Ribosomes are free in the cytoplasm (Fig. 53 & 54).

Fenvalerate treated subjects (5mg/kg body wt./60 days) showed long structure of abnormal nucleus. Outer and inner membranes of the nucleus were ruptured. The mitochondrial outer membranes were ruptured. The cristae are very light. In some mitochondria cristae were not observed. Endoplasmic reticulum found was long but discontinuous. Ribosomes were free in the cytoplasm. Mitochondrial matrix got mixed with cytoplasm (Fig. 55 & 56).

Fenvalerate (10mg/kg body wt./60 days) treated tissues showed abnormal nuclear shape. Outer membrane breakage observed in the nucleus. The mitochondrial outer membranes were ruptured. The mitochondrial matrix was mixed with cytoplasm. Endoplasmic reticulum was long but discontinuous. Ribosomes were free in the cytoplasm. Cristae are light (Fig. 57 & 58).
Fenvalerate treated tissues (PSVT/kg body wt./60 days) showed mild effect in nucleus. Most of the mitochondrial membranes both inner and outer were ruptured. The cristae are very light. Some of them were even not visible properly. In endoplasmic reticulum breakages were observed. Ribosomes were found free in the cytoplasm (Fig. 59 & 60).

**Control Group**

The result showed in the micrograph, the nucleus is composed of both outer and inner membrane were present. The nucleolus are present in the nucleus. The nucleus membrane continuous with endoplasmic reticulum. No mitochondrial membrane breakage were found. Ribosomes were attached with endoplasmic reticulum (Fig. 122 & 124).

**Deformatives in kidney tissues**

**Organocloride**

Endosulfan treated subjects (5mg/kg body wt./30 days) showed nuclear abnormality. The outer membranes were slightly ruptured but cristae are very less and light. Endoplasmic reticulum found long structure but discontinuous. Ribosomes are attached with endoplasmic reticulum (Fig. 61 & 62).

Endosulfan treated subjects (10mg/kg body wt./30 days) showed abnormal shape of nucleus. In some portion of nucleus outer membrane were
ruptured. Mitochondrial outer and inner membrane were ruptured. The vacuole present inside mitochondria. The endoplasmic reticulum were found very light. Ribosomes were free in the cytoplasm (Fig. 63 & 64).

Endosulfan treated subjects (PSVT/kg body wt./30 days) showed mild effect on nucleus. The mitochondrial membrane breakage observed. Endoplasmic reticulum are regularly arranged. The ribosomes are set free in the cytoplasm (Fig. 65 & 66).

Endosulfan treated subjects (5mg/kg body wt./60 days) showed abnormal shape of nucleus. The mitochondrial membrane both inner & outer was ruptured. Cristae were not observed. Endoplasmic reticulum was found very light structure. Ribosomes were free in the cytoplasm (Fig. 67 & 68).

Endosulfan treated subjects (10mg/kg body wt./60 days) showed abnormal shape of nucleus. In some portion outer membrane disappeared. The mitochondrial membrane were ruptured, both inner and outer vacuole formation inside the mitochondria. Endoplasmic reticulum structures were very light and discontinuous. Ribosomes were totally free in the cytoplasm (Fig. 69 & 70).

Endosulfan treated subjects (PSVT/kg body wt./60 days) showed abnormal shape of nucleus. In some portion of nucleus nuclear membrane were ruptured. The outer and inner membrane of the mitochondria was ruptured. Vacuole formation inside the mitochondria was ruptured. No cristae were
observed inside the mitochondria. Endoplasmic reticulum found very light structure. The ribosomes are free in the cytoplasm (Fig. 71 & 72).

**Organophosphate**

Chlorpyriphos treated subjects (5mg/kg body wt./30 days) showed abnormal shape of nucleus of the kidney tissue in albino mice. In nucleus outer and inner membrane were ruptured. The mitochondrial membranes were ruptured and matrix mix with cytoplasm. There was no cristae in the mitochondrial structure. The endoplasmic reticulum discontinuous structure were found. Ribosomes are free in the cytoplasm (Fig. 73 & 74).

Chlorpyriphos treated subjects (10mg/kg body wt./30 days) showed abnormal shape of nucleus. Outer wall were ruptured in the nucleus. Inside the mitochondria vacuole was formed. Mitochondrial membrane both inner and outer were ruptured. The endoplasmic reticulum were breakage in maximum site. The ribosomes were set free in the cytoplasm (Fig. 75 & 76).

Chlorpyriphos treated subjects (PSVT/kg body wt./30 days) showed the mild effect in nucleus. Mitochondrial outer and inner membrane were ruptured. Vacuole present in the mitochondria. Endoplasmic reticulum are long and regular. Ribosomes are attached with endoplasmic reticulum (Fig. 77 & 78).

Chlorpyriphos treated subjects (5mg/kg body wt./60 days) showed triangular shape of nucleus. Outer membranes were ruptured in one side of the
nucleus. The outer and inner membrane of the mitochondria was ruptured. The endoplasmic reticulum was long but discontinuous. Few ribosomes attached with the endoplasmic reticulum, rest are free in the cytoplasm (Fig. 79 & 80).

Chlorpyriphos treated subjects (10mg/kg body wt./60 days) showed nucleus abnormality of the liver in albino mice. Mitochondrial membrane both inner and outer membrane were distorted. No cristae were observed inside the mitochondria. Vacuole formation inside the mitochondria. Endoplasmic reticulum structure were few number and discontinuous. Ribosomes were free in the cytoplasm (Fig. 81 & 82).

Chlorpyriphos treated subjects (PSVT/kg body wt./60 days) showed abnormal nucleus shape of liver in albino mice. The mitochondrial membrane were ruptured. Long and regular structure of endoplasmic reticulum with ribosomal attachment were observed (Fig. 83 & 84).

Malathion treated subjects (5mg/kg body wt./30 days) showed abnormal shape of nucleus. Mitochondrial outer and inner membrane breakage found. Cristae are very less. Endoplasmic reticulum observed irregular. The ribosomes are attached with endoplasmic reticulum (Fig. 85 & 86).

Malathion treated subjects (10mg/kg body wt./30 days) showed abnormal size of nucleus. In few number of mitochondrial outer and inner membrane were ruptured. There were no cristae observed inside the mitochondria. The endoplasmic reticulum structure were very light. The
ribosomes are free in the cytoplasm. Vacuole are observed inside the mitochondria (Fig. 87 & 88).

Malathion treated subjects (PSVT/kg body wt./30 days) showed mild effect on nucleus. Very few mitochondrial membrane were ruptured. Cristae are not observed inside the mitochondria. Endoplasmic reticulum was observed but breakage are found. Few ribosomes were attached with endoplasmic reticulum and rest is free in the cytoplasm (Fig. 89 & 90).

Malathion treated subjects (5mg/kg body wt./60 days) showed abnormal nuclear shape of liver in albino mice with membrane ruptured. Mitochondrial outer and inner membrane were ruptured. No cristae were observed inside the mitochondria. Endoplasmic reticulum was long but breakage observed in it. Ribosomes were set free in the cytoplasm (Fig. 91 & 92).

Malathion treated subjects (10mg/kg body wt./60 days) showed abnormal shape of nucleus with membrane breakage. The mitochondrial membrane was ruptured totally. The matrix comes out and mixes with cytoplasm. Vacuole formed inside the mitochondria. Endoplasmic reticulum were long and very light structure were observed. Ribosomes were free in the cytoplasm (Fig. 93 & 94).

Malathion treated subjects (PSVT/kg body wt./60 days) showed abnormal nuclear shape of liver in albino mice with membrane breakage. The outer membrane ruptured in the mitochondria. No cristae were found inside
the mitochondria. Vacuole formed inside the mitochondria. Endoplasmic structures were found less and discontinuous. Ribosomes were free in the cytoplasm (Fig. 95 & 96).

**Synthetic pyrethroids**

Cypermethrin treated subjects (5mg/kg body wt./30 days) showed abnormal triangular shape of nucleus. In some portion of nucleus outer membrane were ruptured. The mitochondrial outer and inner membrane was ruptured. The two or three number of cristae was present inside the mitochondria. Endoplasmic reticulum structure were found in very less number and breakage observed. The ribosomes were free in the cytoplasm (Fig. 97 & 98).

Cypermethrin treated subjects (10mg/kg body wt./30 days) showed abnormal shape of nucleus. In some portion outer membrane were ruptured. In mitochondria all cristae were disappeared, vacuole present inside the mitochondria. Endoplasmic structures were found in one side very prominent with ribosome attached with it and other side free ribosomes were observed (Fig. 99 & 100).

Cypermethrin treated subjects (PSVT/kg body wt./30 days) showed abnormal shape of nucleus with two nucleolus. The mitochondrial membrane ruptured found in few cases. Cristae are very less in number. Endoplasmic
reticulum found discontinuous with ribosomes attached in it. Maximum number of ribosomes were free in the cytoplasm (Fig. 101 & 102).

Cypermethrin treated subjects (5mg/kg body wt./60 days) showed abnormal shape of nucleus. The nucleus observed finger like projections. Nuclear membrane was ruptured. Mitochondrial membrane, both inner and outer was ruptured. The vacuole formed inside the mitochondria. No cristae were found inside the mitochondria. Endoplasmic reticulum were prominent with ribosomes attached to it (Fig. 103 & 104).

Cypermethrin treated subjects (10mg/kg body wt./60 days) showed abnormal nuclear shape of liver in albino mice. In one side outer membrane were disappeared. Mitochondrial membranes both inner & outer were totally ruptured. Vacuole formed inside the mitochondria. The mitochondrial matrix were come out and mix with cytoplasm. The endoplasmic reticulum were found but breakage were observed. Ribosomes were free in the cytoplasm (Fig. 105 & 106).

Cypermethrin treated subjects (PSVT/kg body wt./60 days) showed abnormal shape of nucleus. Mitochondrial outer membranes were ruptured. No cristae were found inside the mitochondria. Vacuole formed inside the mitochondria. Endoplasmic reticulum structures were observed very less in number and very light. Ribosomes were free in the cytoplasm (Fig. 107 & 108).
Fenvalerate treated subjects (5mg/kg body wt./30 days) showed abnormal shape of nucleus. The mitochondrial membrane was ruptured. No cristae were found inside the mitochondria. Endoplasmic reticulum found discontinuous with ribosomes attached to it. Maximum number of ribosomes was free in the cytoplasm (Fig. 109 & 110).

Fenvalerate treated subjects (10mg/kg body wt./30 days) showed triangular shape of nucleus. The mitochondrial membrane ruptured was observed. Vacuoles were found inside the mitochondria. Endoplasmic reticulum found very light structure. Ribosomes are free in the cytoplasm (Fig. 111 & 112).

Fenvalerate treated subjects (PSVT/kg body wt./30 days) showed mild effect in the shape of nucleus. Few number of mitochondrial membrane ruptured were found. The endoplasmic reticulum structures were long but discontinuous with ribosomes. Maximum number of ribosomes was free in the cytoplasm. There was no cristae observed inside the mitochondria (Fig. 113 & 114).

Fenvalerate treated subjects (5mg/kg body wt./60 days) showed abnormal nuclear shape of liver tissue in albino mice. The outer and inner membrane of mitochondria was ruptured. The mitochondrial matrix was mix with cytoplasm. The endoplasmic reticulum was observed less in number. The ribosomes are free in the cytoplasm (Fig. 115 & 116).
Fenvalerate treated subjects (10mg/kg body wt./60 days) showed abnormal nuclear shape of liver in albino mice. Outer membrane of nucleus disappeared totally. Mitochondrial membrane, both outer and inner was distorted. Vacuole formed inside the mitochondria. Endoplasmic reticulum were prominent in one side but in other side totally disappeared. The ribosomes were free in the cytoplasm (Fig. 117 & 118).

Fenvalerate treated subjects (PSVT/kg body wt./60 days) showed mild effect in nuclear shape. The mitochondrial outer and inner membranes were ruptured. Vacuoles were formed inside the mitochondria. All cristae were disappeared. Endoplasmic reticulum structures were very light. Ribosomes were free in the cytoplasm (Fig. 119 & 120).

**Control Group**

In the untreated/ control group, the nucleus was seen composed of both outer and inner membrane. The nuclear membrane was continuous with endoplasmic reticulum. No membrane breakage was found inside the mitochondria. Ribosomes were attached with endoplasmic reticulum (Fig. 121 & 123).

**DISCUSSION**

Transmission electron microscopic studies revealed various forms of nuclear abnormalities shape, Abnormal mitochondria shape, nuclear membrane breakage, distortion of outer and inner membrane of mitochondria,
vacuolization of mitochondria, breakage of endoplasmic reticulum etc. These all are the effects caused by the pesticides toxicity in the liver & kidney cell of different groups of experimental albino mice studied. The result obtained from cytological study of liver & kidney cells of albino mice may be useful in understanding the physiological problems which results from pesticides toxicity.

It is well known that the abnormalities in liver & kidney cell shape are of considerable physiological importance. Liver cell changes in shape indicate that cell necroses, toxic cell swelling, fatty infiltration were observed (Pathol, 1985). The other chronic effects are reduction in the growth rate, liver enlargement and an increase in the activity of some enzyme in the liver (NRCC, 1986; Mueller et al., 1990). Acute & sub acute studies have been shown that the main effects of synthetic pyrithroids are neurotoxicity at high doses and liver hypertrophy (Litchfield, 1985 & Litchfield, 1983) in number of Mutagenuity studies shows the ability to cause genetic damage by cypermethrin and fenvalerate (Litchfield, 1985; Catind et al., 1989; RPTC, 1990). The most common disease associated with a palpable and enlarged liver include metastatic cancer, lymphoma, longestive heart failure, alcohole hepatitis. In Metastatic or primary cancer, the liver cells become infiltrated with deposits of cancer cells that can grow rapidly. Hepatitis or chemical cause of fatty liver lead to liver enlargement (Douglas, 1990). The breakage & fusion of the nucleus indicate the
mitotic disturbance (Zink et al., 2004). Variation in size & shape reflecting abnormal cell division and maturation. Abnormalities in nuclear morphology are frequently observed in malignant tissues (Zink et al., 2004). It is clear that the relation between abnormal nuclear shape & chromosomal instability was found in tumor cells. Nuclear morphology was also evaluated in fibroblast & an osteosarcoma cell line exposed to irradiation. A correlation was found between the frequency of abnormalities is nuclear shape of liver and unstable chromosomes (Gregory, 2005) DNA content influences the volume of the nuclear, which in turn influences the size of the cell (Cavalier & Smith, 2005; Gregory, 2005). DNA may affects nuclear volume, because the size of the nuclear could be directly proportional to amount of DNA and resulted cell volume increases (Cavalier-Smith, 2005; Jovtchev et al., 2006; Jorgensen et al., 2007; Neumann & Nunse, 2007). An abnormal shape is also associated with Cancer (Zink et al., 2004). The inactivation of proteins that are associated with endoplasmic reticulum affects nuclear shape (Higashio et al., 2000; Matynia et al., 2002). The abnormal nuclear shape is of the key diagnostic tools used in identifying cancerous cells. The nuclear shape, cellular transformation nuclear morphology leads to change in chromosome organization, which in turn can affect gene expression (He et al., 2008). The change in nuclear shape in concern cells facilitates the formation of metastases because of reduced nuclear stiffness, which could increase the ability of transformed cells to penetrate tissue (Dahl et
A defect in nuclear shape can not only from reduced levels of lamina proteins, but also from aberrant processing. The abnormalities in nuclear shape is the rigidity of the nuclear & in chromatin recognition and thereby affect gene expression (Capell et al., 2005; Glynn & Gloven, 2005; Mallampalli et al., 2005; Toth et al., 2005). Mitochondrial abnormalities, effects in electron transport activities resulted typical motor neuron disease observed (Comi et al., 1998).

Neuropathological studies of mice showed that the mitochondrial vacuolization is an early pathological feature (Canto & Gurney 1994; Wong et al., 1995). Mitochondrial vacuolization proved a rapid phase of motor weakness and loss of motor neurons in mice (Gurrey, 1994). A giant mitochondria are responsible for low oxygen pressure in cells (Van, 2002). Autophagic cell death is morphologically characterized by an accumulation of antophagic vacuole (Green & Reed, 1998). Mitochondria are essential in controlling specific apoptosis pathways (Green & Reed, 1998). The mitochondrial abnormalities are mainly oxygen deprivation. Inhibitors of respirations and phosphorylation in a animal cells (Beneiter & Voth 1983; Mankova et al., 1990). Consumption of pesticides shows the biochemical & histologyical alternation in liver mitochondria of albinomice (Koh et al., 2009).

Verma et al. (1978) reported that inhibition of Mg, Na, K and ATPase in a high concentration of aldrine and dieldrin in homogenate of kidney tissue was observed as a result of possible alteration of membrane configuration caused by
pesticides. Kiran and Varma (1990) reported that erythrocyte membrane-associated Na-K ATPase and Mg ATPase activities in rat were significantly decreased as a result of oral administration of 12.5 mg/kg/day body weight of Endosulfan for 4 days. Furthermore, membranes of endoplasmic reticulum and mitochondria, which are sensitive to free radicals, are rich with unsaturated fatty acid (McCord and Fridovich, 1978). It is believed that the effect on unsaturated fatty acids of oxygen free radicals can cause lipid peroxidative injury in membranes (Fridovich, 1978). In the micrographs, the presence of widespread free ribosomes in the cytoplasm of kidney and liver cells is seen. Therefore, it might be thought that widespread free ribosomes in the cytoplasm may be related with toxic effect of Endosulfan, chloropyriphos, malathion, cypermethrin, fenvalerate and pesticide sprayed vegetables treatment. Furthermore, the presence of membranous structure and lipofuscin granules in the cytoplasm of some proximal convoluted tubul cells may reflect the probable injury caused by oxidative effect. Poovala et al. (1999) suggested that organophosphate-induced oxidative stress may play a role in injuring of tubular cells in kidney. Singh and Pandey (1989) reported that the effect of this insecticide on kidney may be related with lipid peroxidative damage of the microsomal membrane. In this study, it is shown that the nuclear mitochondrial and endoplasmic reticulum structure was also affected by administration of all these pesticides. The presence of prominent degeneration in the nuclear
membrane of both liver and kidney were striking. This might indicate the toxic effect of these insecticides. Walker et al. (1983) suggested that the cell surface changes in hepatocytes in administration of acetaminophen may develop as a result of dysfunction of the cytoskeleton. In addition, cellular ATP was decreased. They suggested that oxidative stress and a decrease in the level of cellular ATP may result in bleb formation and damage to the cytoskeleton.

Finally, the effect of the Endosulfan, chlorpyriphos, malathion, cypermethrin, fenvalerate and pesticide sprayed vegetables treatment might cause serious problem in health of an organisms. Furthermore, the membrane distortion in nucleus, mitochondria and discontinuation of endoplasmic reticulum structure and numerous free ribosomes in the cytoplasm was observed in liver and kidney cells only for toxic effect of pesticides. Thus, observing changes in kidney and liver may be thought that oxidative stress may play a role to the mediator in changing configuration of cell membrane and seem to account for the morphologic alteration of kidney and liver in albino mice.
Fig. 1: Effect of Endosulfan showing abnormal nuclear shape of liver tissue in albino mice (5mg/kg body wt./30 days)

Fig. 2: Mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of liver tissue in albino mice (5mg/kg body wt./30 days)

Fig. 3: Effect of Endosulfan showing mitochondrial membrane distortion (M), vacuole (V), free ribosome (R), breakage endoplasmic reticulum (ER) of liver tissue in albino mice (10mg/kg body wt./30 days)

Fig. 4: Abnormal nuclear shape of liver tissue in albino mice (10mg/kg body wt./30 days)
Fig. 5: Effect of Endosulfan showing abnormal nuclear shape of liver tissue in albino mice (PVST/kg body wt./30 days)

Fig. 6: Mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of liver tissue in albino mice (PVST/kg body wt./30 days)

Fig. 7: Effect of Endosulfan showing mitochondrial membrane distortion (M), vacuole (V), free ribosome (R), breakage endoplasmic reticulum (ER) of liver tissue in albino mice (5mg/kg body wt./60 days)

Fig. 8: Abnormal nuclear shape of liver tissue in albino mice (5mg/kg body wt./60 days)
Fig. 9: Effect of Endosulfan showing abnormal nuclear shape of liver tissue in albino mice (10mg/kg body wt./60 days)

Fig. 10: Mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of liver tissue in albino mice (10mg/kg body wt./60 days)

Fig. 11: Effect of Endosulfan showing abnormal nuclear shape of liver tissue in albino mice (PVST/kg body wt./60 days)

Fig. 12: Mitochondrial membrane distortion (M), vacuole (V), free ribosome (R), breakage endoplasmic reticulum (ER) of liver tissue in albino mice (PVST/kg body wt./60 days)
Fig. 13: Effect of Chloropyriphos showing abnormal nuclear shape of liver tissue in albino mice (5mg/kg body wt./30 days)

Fig. 14: Mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of liver tissue in albino mice (5mg/kg body wt./30 days)

Fig. 15: Effect of Chloropyriphos showing mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of liver tissue in albino mice (10mg/kg body wt./30 days)

Fig. 16: Abnormal nuclear shape of liver tissue in albino mice (10mg/kg body wt./30 days)
Fig. 17: Effect of Chloropyriphos showing abnormal nuclear shape of liver tissue in albino mice (PSVT/kg body wt./30 days)

Fig. 18: Mitochondrial membrane distortion (M), vacuole (V), free ribosome (R), breakage endoplasmic reticulum (ER) of liver tissue in albino mice (PSVT/kg body wt./30 days)

Fig. 19: Effect of Chloropyriphos showing mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of liver tissue in albino mice (5mg/kg body wt./60 days)

Fig. 20: Abnormal nuclear shape of liver tissue in albino mice (5mg/kg body wt./60 days)
Fig. 21: Effect of Chloropyriphos showing abnormal nuclear shape of liver tissue in albino mice (10mg/kg body wt./60 days)

Fig. 22: Mitochondrial membrane distortion (M), vacuole (V), free ribosome (R), breakage endoplasmic reticulum (ER) of liver tissue in albino mice (10mg/kg body wt./60 days)

Fig. 23: Effect of Chloropyriphos showing abnormal nuclear shape of liver tissue in albino mice (PSVT/kg body wt./60 days)

Fig. 24: Mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of liver tissue in albino mice (PSVT/kg body wt./60 days)
Fig. 25: Effect of Malathion showing abnormal shape nucleus of liver tissue in albino mice (5mg/kg body wt./30 days)

Fig. 26: Mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of liver tissue in albino mice (5mg/kg body wt./30 days)

Fig. 27: Effect of Malathion showing mitochondrial membrane distortion (M), Vacuole (V), free ribosome (R), breakage endoplasmic reticulum (ER) of liver tissue in albino mice (10mg/kg body wt./30 days)

Fig. 28: Abnormal shape of nucleus of liver tissue in albino mice (10mg/kg body wt./30 days)
Fig. 29: Effect of Malathion showing abnormal shape of nucleus of liver tissue in albino mice (PSVT/kg body wt./30 days)

Fig. 30: Mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of liver tissue in albino mice (PSVT/kg body wt./30 days)

Fig. 31: Effect of Malathion showing abnormal nuclear shape with mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of liver tissue in albino mice (5mg/kg body wt./60 days)

Fig. 32: Mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of liver tissue in albino mice (5mg/kg body wt./60 days)
Fig. 33: Effect of Malathion showing abnormal shape of nucleus of liver tissue in albino mice (10mg/kg body wt./60 days)

Fig. 34: Mitochondrial membrane distortion (M), Vacuole (V), free ribosome (R), breakage endoplasmic reticulum (ER) of liver tissue in albino mice (10mg/kg body wt./60 days)

Fig. 35: Effect of Malathion showing abnormal shape of nucleus of liver tissue in albino mice (PSVT/kg body wt./60 days)

Fig. 36: Mitochondrial membrane distortion (M), Vacuole (V), free ribosome (R), breakage endoplasmic reticulum (ER) of liver tissue in albino mice (PSVT/kg body wt./60 days)
Fig. 37: Effect of Cypermethrin showing abnormal shape of nucleus of liver tissue in albino mice (5mg/kg body wt./30 days)

Fig. 38: Mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of liver tissue in albino mice (5mg/kg body wt./30 days)

Fig. 39: Effect of Cypermethrin showing abnormal shape of nucleus of liver tissue in albino mice (10mg/kg body wt./30 days)

Fig. 40: Mitochondrial membrane distortion (M), Vacuole (V), free ribosome (R), breakage endoplasmic reticulum (ER) of liver tissue in albino mice (10mg/kg body wt./30 days)
Fig. 41: Effect of Cypermethrin showing abnormal shape of nucleus of liver tissue in albino mice (PSVT/kg body wt./30 days)

Fig. 42: Mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of liver tissue in albino mice (PSVT/kg body wt./30 days)

Fig. 43: Effect of Cypermethrin showing mitochondrial membrane distortion (M), Vacuole (V), free ribosome (R), breakage endoplasmic reticulum (ER) of liver tissue in albino mice (5mg/kg body wt./60 days)

Fig. 44: Abnormal nuclear shape of liver tissue in albino mice (5mg/kg body wt./60 days)
Fig. 45 : Effect of Cypermethrin showing mitochondrial membrane distortion (M), Vacuole (V), free ribosome (R), breakage endoplasmic reticulum (ER) of liver tissue in albino mice (10mg/kg body wt./60 days)

Fig. 46 : Abnormal nuclear shape of liver tissue in albino mice (10mg/kg body wt./60 days)

Fig. 47 : Effect of Cypermethrin showing mitochondrial membrane distortion (M), Vacuole (V), free ribosome (R), breakage endoplasmic reticulum (ER) of liver tissue in albino mice (PSVT/kg body wt./60 days)

Fig. 48 : Abnormal nuclear shape of liver tissue in albino mice (PSVT/kg body wt./60 days)
Fig. 49: Effect of Fenvalerate showing mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of liver tissue in albino mice (5mg/kg body wt./30 days)

Fig. 50: Abnormal nuclear shape of liver tissue in albino mice (5mg/kg body wt./30 days)

Fig. 51: Effect of Fenvalerate showing mitochondrial membrane distortion (M), Vacuole (V), free ribosome (R), breakage endoplasmic reticulum (ER) of liver tissue in albino mice (10mg/kg body wt./30 days)

Fig. 52: Abnormal nuclear shape of liver tissue in albino mice (10mg/kg body wt./30 days)
Fig. 53: Effect of Fenvalerate showing abnormal nuclear shape of liver tissue in albino mice (PSVT/kg body wt./30 days)

Fig. 54: Mitochondrial membrane distortion (M), Vacuole (V), free ribosome (R), breakage endoplasmic reticulum (ER) of liver tissue in albino mice (PSVT/kg body wt./30 days)

Fig. 55: Effect of Fenvalerate showing abnormal nuclear shape of liver tissue in albino mice (5mg/kg body wt./60 days)

Fig. 56: Mitochondrial membrane distortion (M), Vacuole (V), free ribosome (R), breakage endoplasmic reticulum (ER) of liver tissue in albino mice (5mg/kg body wt./60 days)
Fig. 57: Effect of Fenvalerate showing abnormal shape of nucleus of liver tissue in albino mice (10mg/kg body wt./60 days)

Fig. 58: Mitochondrial membrane distortion (M), Vacuole (V), free ribosome (R), breakage endoplasmic reticulum (ER) of liver tissue in albino mice (10mg/kg body wt./60 days)

Fig. 59: Effect of Fenvalerate showing abnormal shape of nuclear of liver tissue in albino mice (PVST/kg body wt./60 days)

Fig. 60: Mitochondrial membrane distortion (M), Vacuole (V), free ribosome (R), breakage endoplasmic reticulum (ER) of liver tissue in albino mice (PVST/kg body wt./60 days)
Fig. 61: Effect of Endosulfan showing abnormal shape of nucleus of kidney tissue in albino mice (5mg/kg body wt./30 days)

Fig. 62: Mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of kidney tissue in albino mice (5mg/kg body wt./30 days)

Fig. 63: Effect of Endosulfan showing abnormal shape of nuclear of kidney tissue in albino mice (10mg/kg body wt./30 days)

Fig. 64: Mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of kidney tissue in albino mice (10mg/kg body wt./30 days)
Fig. 65: Effect of Endosulfan showing abnormal shape of nucleus of kidney tissue in albino mice (PSVT/kg body wt./30 days)

Fig. 66: Mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of kidney tissue in albino mice (PSVT/kg body wt./30 days)

Fig. 67: Effect of Endosulfan showing abnormal shape of nuclear of kidney tissue in albino mice (5mg/kg body wt./60 days)

Fig. 68: Mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of kidney tissue in albino mice (5mg/kg body wt./60 days)
Fig. 69: Effect of Endosulfan showing abnormal shape of nucleus of kidney tissue in albino mice (10mg/kg body wt./60 days)

Fig. 70: Mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of kidney tissue in albino mice (10mg/kg body wt./60 days)

Fig. 71: Effect of Endosulfan showing mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of kidney tissue in albino mice (PSVT/kg body wt./60 days)

Fig. 72: Abnormal nuclear shape of kidney tissue in albino mice (PSVT/kg body wt./60 days)
Fig. 73: Effect of Chlorpyriphos showing mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of kidney tissue in albino mice (5mg/kg body wt./30 days)

Fig. 74: Abnormal shape of nucleus (N), mitochondrial membrane distortion (M) of kidney tissue in albino mice (5mg/kg body wt./30 days)

Fig. 75: Effect of Chlorpyriphos showing mitochondrial membrane distortion (M), Vacuole (V), free ribosome (R), breakage endoplasmic reticulum (ER) of kidney tissue in albino mice (10mg/kg body wt./30 days)

Fig. 76: Abnormal shape of nucleus (N), mitochondrial membrane distortion (M), breakage endoplasmic reticulum (ER) of kidney tissue in albino mice (10mg/kg body wt./30 days)
Fig. 77: Effect of Chlorpyriphos showing abnormal shape of nucleus (N), mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of kidney tissue in albino mice (PVST/kg body wt./30 days)

Fig. 78: Mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of kidney tissue in albino mice (PVST/kg body wt./30 days)

Fig. 79: Effect of Chlorpyriphos showing mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of kidney tissue in albino mice (5mg/kg body wt./60 days)

Fig. 80: Abnormal shape of nucleus (N) of kidney tissue in albino mice (5mg/kg body wt./60 days)
Fig. 81: Effect of Chlorpyriphos showing abnormal shape of nucleus (N) of kidney tissue in albino mice (10mg/kg body wt./60 days)

Fig. 82: Mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of kidney tissue in albino mice (10mg/kg body wt./60 days)

Fig. 83: Effect of Chlorpyriphos showing mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of kidney tissue in albino mice (PVST/kg body wt./60 days)

Fig. 84: Abnormal shape of nucleus (N), mitochondrial membrane distortion (M) of kidney tissue in albino mice (PVST/kg body wt./60 days)
Fig. 85: Effect of Malathion showing mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of kidney tissue in albino mice (5mg/kg body wt./30 days).

Fig. 86: Abnormal shape of nucleus (N) of kidney tissue in albino mice (5mg/kg body wt./30 days).

Fig. 87: Effect of Malathion showing abnormal shape of nucleus (N) kidney tissue in albino mice (10mg/kg body wt./30 days).

Fig. 88: Mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of kidney tissue in albino mice (10mg/kg body wt./30 days).
Fig. 89: Effect of Malathion showing abnormal shape of nucleus (N), mitochondrial membrane distortion (M) of kidney tissue in albino mice (PSVT/kg body wt./30 days)

Fig. 90: Mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of kidney tissue in albino mice (PSVT/kg body wt./30 days)

Fig. 91: Effect of Malathion showing abnormal shape of nucleus (N) of kidney tissue in albino mice (5mg/kg body wt./60 days)

Fig. 92: Mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of kidney tissue in albino mice (5mg/kg body wt./60 days)
Fig. 93: Effect of Malathion showing mitochondrial membrane distortion (M), Vacuole (V), free ribosome (R), breakage endoplasmic reticulum (ER) of kidney tissue in albino mice (10mg/kg body wt./60 days)

Fig. 94: Abnormal shape of nucleus (N), mitochondrial membrane distortion (M) of kidney tissue in albino mice (10mg/kg body wt./60 days)

Fig. 95: Effect of Malathion showing mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of kidney tissue in albino mice (PSVT/kg body wt./60 days)

Fig. 96: Abnormal shape of nucleus (N), mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of kidney tissue in albino mice (PSVT/kg body wt./60 days)
Fig. 97: Effect of Cypermethrin showing abnormal shape of nucleus (N), mitochondrial membrane distortion (M), breakage endoplasmic reticulum (ER), free ribosome (R) of kidney tissue in albino mice (5mg/kg body wt./30 days).

Fig. 98: Mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of kidney tissue in albino mice (5mg/kg body wt./30 days).

Fig. 99: Effect of Cypermethrin showing abnormal shape of nucleus (N), mitochondrial membrane distortion (M) of kidney tissue in albino mice (10mg/kg body wt./30 days).

Fig. 100: Mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of kidney tissue in albino mice (10mg/kg body wt./30 days).
Fig. 101: Effect of Cypermethrin showing abnormal shape of nucleus (N), mitochondrial membrane distortion (M) of kidney tissue in albino mice (PSVT/kg body wt./30 days)

Fig. 102: Mitochondrial membrane distortion (M), free ribosome (R) of kidney tissue in albino mice (PSVT/kg body wt./30 days)

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Fig. 119: Effect of Fenvalerate showing mitochondrial membrane distortion (M), free ribosome (R), breakage endoplasmic reticulum (ER) of kidney tissue in albino mice (PSVT/kg body wt./60 days).

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Chapter 5

5. C. PATHOLOGICAL STUDIES
ON HUMAN BLOOD

INTRODUCTION

Pesticide is a general term for substances, which are used to poison pests (weeds, insects, molds, rodents etc.) The pesticides most acutely dangerous for humans are insecticides and rodenticides. Synthetic pesticides have been population with farmers, because of their widespread availability, simplicity in application, efficacy and economic returns. But they also have huge environmental costs.

After India’s Green Revolution started, the consumption of pesticides in India has increased several hundred folds, from 154 MT in 1954 to 88,000 MT in 2000-2001. According to industry estimates, the pesticides use has high growth potential in India.

Notwithstanding the fact that overall consumption of pesticides in India as a whole is low than that used in the development countries of the world, there is still a widespread contamination of water, soil and water pesticides residues. In India, among different states maximum consumption of pesticides 1999-2000 was in Uttar Pradesh (7459 MT) followed by Punjab.
(6972 MT), Haryana (5025 MT), Andra Pradesh (4054 MT), Gujarat (3646 MT). Leading pesticides used in India monocrotophos (10700 MT – highest consumed), acephate (6400 MT), endosulfan (5600 MT) and chlorpyrifos (5000 MT – fourth highest consumed) (Sources : Pesticides Information, Volume XXVIII, No. 3, October – December 2002). Across the globe pesticides have been found in human blood, urine, breast milk, semen, adipose tissue, amniotic fluid, infant meconium and umbilical cord blood. The Blood parameters have been used as Sensitive indication of stress in mammals exposed to different water pollutants & toxicants, such as metal, biocides, pesticides, chemical industries effluents etc. The present work has been undertaken keeping the above in the backdrop.

**REVIEW OF LITERATURE**

Cumulative exposure to pesticides may come from flood, water, air, dust, soil etc. Pesticides can be absorbed though skin contact, inhalation or accidental ingestion. Farm workers came into direct contact with pesticides at work as well and are occupationally exposed to them. When a person is exposed to pesticides, body’s detoxification mechanisms are activated. Some pesticides are metabolized into different chemicals and excreted and some are stored in fatty tissues in the body. Body burden data from analysis of blood provides evidence of exposure to chemicals stored in our body. There are several studies international and national on pesticide residues found in
blood samples. The pyrithoids insecticides are commonly used to control insect pests & represent about 30% (Amdun et al., 1991) of world insecticides consumption. The variation of blood parameters demonstrated to be sensitive to sublethal concentration of different toxic agent, they can be used for detecting pollutants explosive in the environment (National Research Council, 1989). A decrease in important blood parameters has been reported during exposure to various pesticides in fishes (Reddy & Chaturved, 1995; Nath 1996, Sexena & Seth, 2002) Changes in Haematological parameters might have been brought about by cypermethrin as an anemic condition due to decreased synthesis of Hb & RBC number in bone marrow cells. So, Haematological alterations may be used for diagnosis in the field to assess pollution related pathological alterations in animals changes of heamatological parameters in prochilodus lineatus exposed to sublethal concentration of cypermethrin (Panma et al., 2007). Is was observed that will the increase of exposure line total erythrocytes (RBC), hemoglobin (Hb, hematocrit (HC) & Mean corpuscular hemoglobin concentration (MCHC) value decreased but Mean corpuscular volume (MVC) & Mean corpuscular hemoglobin (MCH) Values increased. Also (Shenif and Eisa, 1994) it was observed that the sublethal concentration of chloropyifes and recorded histopathological changes, necrosis of hepatocytes. The hepatopancreas is the site of detoxification of all types of toxin and chemicals (Rebent, 2001)
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Stress response is characterize by physiological change and the effect of pollutants on fish is assessed by acute and chronic toxicity tests (Health, 1991. The stress caused by environment pollution, changes the structure of
red and white blood cells (Lesson *et al.*, 1984). Haematological techniques are the most common method to determine the sub-lethal effect of the pollutants (Lesson *et al.*, 1985).

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According to Pamila et al. (1991) the reduction in haemoglobin content in a fish exposed to toxicant could be due to the inhibitory effect of those toxic substance on the enzyme system responsible for synthesis of haemoglobin. The pollutant entering into fish system are slowly eliminated (Newman and Mitz., 1988; James and Sampath, 1996; James et al., 1996), and hence the blood parameters got effected on account of pollutant toxicity.

The pesticides enters the human body and gets accumulated in various organs like liver and kidney (Al-Mohanna, 1994).

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The blood parameters have been used as sensitive indicator of stress animal exposed to different pollutants and toxicants, such as biocides,
pesticides, industrial effluents etc (Singh et al., 2008). Some authors (Reddy and Bashamohidden, 1989; Chauthan et al., 1994; Agarwal and Chaturvedi. 1995 Nath, 1996) have reported a decrease in hematocrit hemoglobin and red blood cells values of some fish after their exposure to insecticides. Sexena and Seth (2002) showed a significant change in the Haematology of the common fresh water fish. The information suggests that Haematological parameters could be used as potential biomarkers of pyrethroid insecticides. A decrease in important blood parameters has been reported during exposure to various pesticides (Reddy and Bashamohideun), 1989; Chauhan, et al. 1994; Agarwal & Caturvedi, 1995 Nath, 1996; Sexena and Seth, 2002). Changes in heamatological parameters might have been brought about by cypermethrin as an anemic condition due to decreased synthesis of Hb & RBC number.

The haematological alternation maybe used for diagnosis in the field to asses pollution related Pathophysiological alternations. Alteration of haematological parameters has been associated with them physiological state and maybe induced directly by genotoxic compounds. Pyrethroid insecticides are commonly used to control insects pets and represent about 30% (Amedun et al., 1991) of world insecticide consumption).

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As large number of chemicals are found as contaminants in the ecosystem, food and water and find their way into the food chain of man. These substances exert their toxic effects depending upon their mode of absorption, distribution, metabolism and excretion. Some of the substances are directly toxic while others cause ill-effects via their metabolites. Environmental chemicals may have slow damaging effect or there may be sudden accidental exposure. The problem of pesticide contamination is particularly alarming in developing countries (like India, China and Mexico), where farmers and their families are unknowingly exposed to these hazardous chemicals during aerial spraying on crops. According to WHO estimates, about 7.5 lakh people are taken ill every year worldwide with pesticide poisoning, half of which occur in the developing countries. Blood is a liquid connective tissue and patho-physiological reflector of the whole body. Blood parameters are important in diagnosis of the status of animal exposed to toxicant. The study reveals that the blood parameters are sensitive indicator of stress in any animal exposed to different toxicants, such as metals, biocides, pesticides, chemical effluents. Pesticides can be absorbed though skin contact, inhalation or accidental ingestion. Farm
workers came into direct contact with pesticides at work as well and are occupationally exposed to them.

The environmental pollution induced by the chemical substances is regarded as a serious problem on the ecosystem. Recently, many people are interest in safety of foods. We focused on two pesticides chemicals methoxychlor and endoculfan presently used in agricultural farms in many countries.

These results suggest that these pesticides affect immune system as well as detoxification tissues. It can be suggested from our present finding that the exposure to these compounds via food may lead to the decline in immune activities of human body, resulting in diseases.

The widespread environmental pollution caused by the chemical substances such as pesticides is a serious problem for creatures including human (McClure et al., 2001; McKinlay et al., 2008; Boobis et al., 2008), various chemical substances entering animal bodies are carried to the organs responsible for detoxification, such as liver and kidney, and excreted. On the other hand, some hydrophobic substances among these chemicals are accumulated in our body (Somogyi and Beck, 1993).

In some previous reports, influence of these pesticides on reproductive system has been studied (Welchons et al., 1999; Bretveld et al., 2007).
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The anaemia might have led to a fall in the red blood cell count,
haemoglobin, and haematocrit volume. Anaemia under any chemical stress.

Similar results with significant reduction of RBCs and Hb% content in
fishes exposed to different heavy metals have been reported previously by
Goel et al., (1985) and Goel and Sharma (1987), high white blood cell counts
indicate damage due to infection of body tissues, severe physical stress, and
as well leukemia. In most cases, abnormal red cell morphology is need Nath

The lymphocytes are reported to be responsible for immune response
while monocytes are partially differentiated end cells, which under
appropriate circumstances develop into mature cells of mononuclear
phagocyte system but are not capable of further division.

Neutrophils and monocytes are important white blood cells to protect
the body, through their elevated phagocyte activity, against bacterial
infection in damaged tissue. Release of neutrophils in to the blood, causing a
disease neutrophilia, is known to occur as a non-specific response to a
variety of stress generally decreases during exposure to pollutants (Nussey
et al., 1995; Svobodova et al., 1994)
According to Pamila et al. (1991) the reduction in haemoglobin content in a fish exposed to toxicant could be due to the inhibitory effect of those toxic substance on the enzyme system responsible for synthesis of haemoglobin. The pollutant entering into fish system are slowly eliminated (Newman and Mitz., 1988; James and Sampath, 1996; James et al., 1996), and hence the blood parameters got effected on account of pollutant toxicity.

The pesticides enters the human body and gets accumulated in various organs like liver and kidney (Al-Mohanna, 1994).

Pollutants induced stress may be due to blood cell injury and disrupted haemoglobin synthesis (Mckin et al., 1970; Gross et al., 1975.

The blood parameters have been used as sensitive indicator of stress animal exposed to different pollutants and toxicants, such as biocides, pesticides, industrial effluents etc (Singh et al., 2008). The Blood parameters have been used as Sensitive indication of stress in mammals exposed to different water pollutants & toxicants, such as metal, biocides, pesticides, chemical industries effluents etc. These chemical substance are the probable in mammal Blood.

MATERIALS AND METHODS

Forty eight (48) blood samples collected from tea garden labourers of Rosekandy Tea Estate, Cachar, Assam, were analyzed. The samples were collected from three groups of workers. 15 blood samples were collected
from the workers who are actively engaged in application of pesticides in the tea field; 16 samples from the factory workers and 17 samples from the labourers who are engaged in the plucking of tea leaves. In the sprayer group, there are 15 males; in factory group 16 males were there, while in the plucking group, there were 5 males and 12 females. All the labourers were between 20 - 53 age group.

The different parameters analyzed were WBC count, RBC count, Haemoglobin (%), Haematocrit (HCT), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Blood Platelet Count (PLT), Red Cell Distribution Width – its Standard Deviation (RDW-SD), Co-efficient of variation of Red Cell Distribution Width (RDW-CV) Platelet Distribution Width (PDW), Mean Platelet Volume(MPV), Platelet-large cell ratio (P-LCR ), Platelet Crit (PCT), Differential leucocyte count (DLC) to observe the various proportions of neutrophils, Lymphocytes, Monocytes, Eosinophils and Basophils. In addition to these, major abnormalities, if any, were studied while doing the pathological analysis of the collected blood samples.

The blood sample was collected from the tea garden labourers of Rosekandy in Eppendorf tubes containing EDTA anticoagulant.

**Total count of RBC**

Total red blood cells (tRBC) were counted using an improved Neubau haemocytometer (Shah and Altindag 2004a). Blood was diluted 1:200 with
Hayem’s fluid (Mishra et al., 1977). Erythrocytes were counted in the loaded haemocytometer chamber and total numbers were reported as $10^6 \mu m^3$ (Wintrobe, 1967).

**Total count of WBC**

Total white blood cells (WBC) were counted using an improved Neubaur haemocytometer (Shah and Altindag 2005; Mgbenka et al., 2003). Blood was diluted 1:20 with Turk’s diluting fluid and placed in haemocytometer 4 large (1 sq. mm) corner squares of the haemocytometer were counted under the microscope (Olympus) at 640 X. The total number of WBC was calculated in $\mu m^3 \times 10^3$ (Wintrobe, 1967).

**Estimation of Haemoglobin**

Hemoglobin (Hb) was determined with a hemoglobin test kit (Diagnova, Ranbaxy, India) using the cyammethemoglobin method. Haemoglobin was determined sperophotometrically (540 nm) using cyanomethemoglobin method. Hct was determined by the microhaematocrit. Technique using capillary tubes by centrifugation at 12000 rpm for 5 minutes.

Mean corpuscular volume (MCV) mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated according to Ranzani-Paiva (1991).
RESULTS

Analysis of blood samples of tea garden labourers belonging to three different groups (viz., spraying group, factory worker group and pluckers group) revealed the following details.

**Hemoglobin Count (Hb%)**: on an average, all the blood samples under study showed anaemic as the common feature. The percentage of anaemic population were 75% among factory workers, 80% among spraying group and 94% among the pluckers (Fig. 6).

**Total Leucocyte Count (TLC)**: Among the labourers working in the spraying group, TLC in all were found to be within normal range; however, among factory workers, 31% were found to be having higher TLC count and among the plucking workers, 6% were found to be below the range, another 6% were above the range, while the remaining 88% were quite within the normal range.

It is needless to mention here that reduction of leucocyte (WBC) makes the person more susceptible to various diseases. However, from the present study, it has been found that all, except one samples, had the TLC level within the range.

**Differential Leucocyte Count (DLC)**: The differential leucocyte count is estimated by analyzing five components of WBC. These are Neutrophils, Lymphocytes, Monocytes, Eosinophils and basophils.
(i) Neutrophils: In a normal healthy adult male, its normal range is 34.0-67.9, while in females, it is 31.0-71.1. In all the blood samples studied under the three groups, 13% in spraying and 6% in plucking groups were found to be below the normal range; while 6% factory workers were having higher neutrophils level. Remaining all was found to be within the standard range. Neutrophils are the key components in the system of defense against infection. An absence or scarcity of neutrophils make the person vulnerable to infection. An increase of neutrophils level signifies acute bacterial infection, while, decrease proportion of neutrophils may signify viral infections (Fig. 1).

(ii) Lymphocyte: In a normal healthy adult male, its normal range is 21.8-53.1, while in females, it is 19.3-51.7. Among all the samples under study, 6% in spraying, 6% in factory and 11% in plucking workers were found to be below the normal range. In Lymphocyte (T cells and B cells) plays a large role in defending the body against diseases. B cells make antibodies that attack bacteria and toxins; while T cells attack body cells themselves when they have been taken over by viruses or have become cancerous. Therefore, the reduction of Lymphocytes in patients deserves appropriate medical attention (Fig. 2).
(iii) Monocytes: The normal range in a healthy male is 5.3-12.2, while in females, it is 4.7-12.5. Among sprayers as well as pluckers, its range was found to be quite within range, but among the factory workers, 6% showed higher monocyte content. Monocytes play an important role in the immune defense, inflammation, and tissue remodeling. An increased percentage of monocytes may indicate chronic inflammatory disease, tuberculosis, various viral infections like mononucleosis, mumps, measles, etc.

(iv) Eosinophils: Its normal range in a healthy adult male is 0.8-7.0, while in females it is 0.7-5.8. Among spraying group of workers all, while 75% among factory workers & 94% among plucking workers showed the higher eosinophil content, and thus eosinophilia appears to be a major and predominant pathological disorder among all the subjects under review. It is an established fact that in areas where parasitic diseases are common, they are the usual cause of eosinophilia (Fig. 3).

Eosinophilia may be primary or secondary. An abnormal increase of hematopoietic stem cell may cause primary eosinophilia, while in secondary eosinophilia, the increased production of eosinophils is a reactive process driven by cytokines, as in the case of allergy.
(v) Basophils: Its normal range in adult male is 0.2-1.2, while in females, it is 0.1-1.2. Among sprayes 13%, while 11% in plucking workers showed basophil level higher than the normal range. However, among factory workers, all showed basophil level within normal range. Basophil level generally increases during infection. Therefore, the persons with higher basophil level deserve further medical attention.

**Blood Platelets (PLT):** The range of blood platelet in normal male is 163-337, while in females, it is 182-369. Among the studied samples, 33% spraying workers showed below the range & 6% above the range, while among factory workers 25% & among pluckers 52% showed platelet count below the normal range. Increase of platelet count has direct correlation with the incidence of cardiovascular problems. Therefore, these aspects deserves appropriate & timely attention. Reduction in platelet count below the normal range may cause uncontrolled bleeding. Therefore, this aspect must be taken care of (Fig. 4).

**Haematocrit (HCT):** In a normal healthy adult male, its normal range is 34.1-44.9, while in females, it is 40.1-51.0. In all the blood sample studied under the three groups, i.e., in spraying 33% were below the normal range, 67% were within the normal range. In the factory worker only 6% were below the range and in plucking, 35% were found to be below the range.
Elevated Haematocrit may indicate dengue fever. Also in Polychthemiavera (PV), a myeloproliferative disorder, the bone marrow produces excess number of red cells; which is associated with elevated Haematocrit. Chronic obstructive pulmonary disease (COPD) may elicit an increased production of red cells (Fig. 5).

Lower Haematocrit can imply significant hemorrhages, as in ectopic pregnancy. This may be a regular case in infants/patients who do not take requisite quantity of iron supplement. Also patients with chronic kidney disease, (where kidney no longer secrete sufficient level of the hormone erythropoietin,) lower level of haemocrite may be found.

**Mean corpuscular volume (MCV):** In a normal adult healthy male, its normal range is 79.0-92.2, while in females, it is 79.4-94.8. Among all the samples under study, in spraying 47% were found to be below the range, 33% within the normal range and 20% were above the range. In factory 6% found to be the above range. While in plucking 29% were found to be below the range and 6.0% were found to be within the range.

There are many possible reasons why the MCV level can be too high, one reason is because of liver disease. Liver cirrhosis can cause high MCV level. Another cause of high MCV is alcohol abuse. Hypothyroidism can cause MCV level to be high.

Another condition that can cause an increased MCV level is Myelofibrosin, in which the normal bone marrow is replaced by fibrous
tissue Reticulocytosis (increased number of reticulocytes in the blood). Also, having too little vitamin B12 or folic acid can cause the MCV to increase. There are many possible reasons, why the MCV level can be low, one reason is because of lead poisoning. Along term decrease in iron in the body can also cause low MCV.

**Mean Corpuscular volume (MCH)**: In a normal healthy adult male 25.7-32.2 and in females 25.6-32.2. In spraying worker group 47% below the range, 53% were within the range. In factory worker group 6.0% were found to be within the range, while in plucking groups 29% below the range and 6.0% were within the range.

**Mean Corpuscular Haemoglobin Concentration (MCHC)**: in a normal healthy male the range is 32.3-36.5 and in female 32.2-35.5. From the blood sample studied among the spraying groups 67% were below the range & 33% were above the range. In factory only 6.0% were below the ranges while in plucking groups 35% were found to be below the range.

**Red Cell Distribution Width**: Its standard Deviation (RDW-SD):- In a normal healthy male the range is 35.1-43.9 & in female, it is 36.4-43.9. In spraying 33% were found to be normal & rest 67% were found to be above the normal range. While in factory worker 6.0% were above range & in plucking groups 17% were found to be within the normal range, 17% were to be the above from the normal range.
Co-efficient of Variation Red Cell Distribution Width (RDW-CV): In a normal healthy adult male the range is 11.6-14.4 & in female, it is 11.7-14.4. In the sprayer groups 33% were found to be normal & 67% were found to be above the normal range. While in factory 6.0% were found to be above the normal range & in plucking groups 6.0% were found to be normal & 29% were found to be above the range.

Fig. 1: Neutrophils shows the percentage

Fig. 2: Lymphocytes shows the percentage
Fig. 3: Eosinophils shows the percentage

Fig. 4: Blood platelets shows the percentage

Fig. 5: Haematocrit shows the percentage
Fig. 6: Haemoglobin shows the percentage

Table 1: Regularly exposed to pesticides (Field Workers)

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Blood sample code</th>
<th>Date of sampling</th>
<th>Name of person</th>
<th>Sex</th>
<th>Age (years)</th>
<th>District</th>
<th>Name of the Place</th>
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Table 2: Regularly exposed to pesticides (Factory Workers)

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<th>Name of person</th>
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<th>Age (years)</th>
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Table 3: Regularly exposed to pesticides (Plucking)

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<td>Ranjan Ree</td>
<td>M</td>
<td>42</td>
<td>Cachar</td>
<td>Rosekandy T.E.</td>
</tr>
<tr>
<td>16</td>
<td>C49</td>
<td>17.10.2009</td>
<td>Sumitra Ree</td>
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<td>20</td>
<td>Cachar</td>
<td>Rosekandy T.E.</td>
</tr>
<tr>
<td>17</td>
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<td>17.10.2009</td>
<td>Menoka Singh</td>
<td>F</td>
<td>35</td>
<td>Cachar</td>
<td>Rosekandy T.E.</td>
</tr>
</tbody>
</table>
Table 4: Parameters show the highest, lowest and mean value of spraying groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Highest</th>
<th>Lowest</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>7.67</td>
<td>4.73</td>
<td>6.182</td>
</tr>
<tr>
<td>RBC</td>
<td>5.84</td>
<td>3.49</td>
<td>4.598</td>
</tr>
<tr>
<td>HGB</td>
<td>15.6</td>
<td>5.2</td>
<td>11.68</td>
</tr>
<tr>
<td>HCT</td>
<td>46.9</td>
<td>20.3</td>
<td>36.866</td>
</tr>
<tr>
<td>MCV</td>
<td>95.8</td>
<td>70.4</td>
<td>80.52</td>
</tr>
<tr>
<td>MCH</td>
<td>31.5</td>
<td>14.4</td>
<td>25.493</td>
</tr>
<tr>
<td>MCHC</td>
<td>33.6</td>
<td>25.6</td>
<td>31.44</td>
</tr>
<tr>
<td>PLT</td>
<td>355</td>
<td>129</td>
<td>179.93</td>
</tr>
<tr>
<td>RDW-SD</td>
<td>55.2</td>
<td>38.8</td>
<td>46.466</td>
</tr>
<tr>
<td>RDW-CV</td>
<td>23.6</td>
<td>12.0</td>
<td>27.986</td>
</tr>
<tr>
<td>PDW</td>
<td>18.5</td>
<td>16.5</td>
<td>17.38</td>
</tr>
<tr>
<td>MPV</td>
<td>14.0</td>
<td>11.1</td>
<td>12.42</td>
</tr>
<tr>
<td>P-LCR</td>
<td>55.0</td>
<td>33.9</td>
<td>43.08</td>
</tr>
<tr>
<td>PCT</td>
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<td>0.17</td>
<td>0.262</td>
</tr>
<tr>
<td>NEUT</td>
<td>3.71</td>
<td>1.36</td>
<td>2.441</td>
</tr>
<tr>
<td>LYMPH</td>
<td>2.96</td>
<td>1.35</td>
<td>1.931</td>
</tr>
<tr>
<td>MONO</td>
<td>0.84</td>
<td>0.29</td>
<td>0.498</td>
</tr>
<tr>
<td>EO</td>
<td>3.03</td>
<td>0.42</td>
<td>1.275</td>
</tr>
<tr>
<td>BASO</td>
<td>0.08</td>
<td>0.01</td>
<td>0.035</td>
</tr>
</tbody>
</table>
Table 5: Parameters show the highest, lowest and mean value of factory groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Highest</th>
<th>Lowest</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>13.48</td>
<td>5.55</td>
<td>8.438</td>
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<tr>
<td>RBC</td>
<td>Same</td>
<td>Same</td>
<td>3.33</td>
</tr>
<tr>
<td>HGB</td>
<td>14.4</td>
<td>9.9</td>
<td>12.55</td>
</tr>
<tr>
<td>HCT</td>
<td>Same</td>
<td>Same</td>
<td>30.8</td>
</tr>
<tr>
<td>MCV</td>
<td>&quot;</td>
<td>&quot;</td>
<td>92.5</td>
</tr>
<tr>
<td>MCH</td>
<td>&quot;</td>
<td>&quot;</td>
<td>29.7</td>
</tr>
<tr>
<td>MCHC</td>
<td>&quot;</td>
<td>&quot;</td>
<td>32.1</td>
</tr>
<tr>
<td>PLT</td>
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<td>56</td>
<td>163.31</td>
</tr>
<tr>
<td>RDW-SD</td>
<td>Same</td>
<td>Same</td>
<td>53.0</td>
</tr>
<tr>
<td>RDW-CV</td>
<td>Same</td>
<td>Same</td>
<td>16.3</td>
</tr>
<tr>
<td>PDW</td>
<td>&quot;</td>
<td>&quot;</td>
<td>-</td>
</tr>
<tr>
<td>MPV</td>
<td>&quot;</td>
<td>&quot;</td>
<td>-</td>
</tr>
<tr>
<td>P-LCR</td>
<td>&quot;</td>
<td>&quot;</td>
<td>-</td>
</tr>
<tr>
<td>PCT</td>
<td>&quot;</td>
<td>&quot;</td>
<td>-</td>
</tr>
<tr>
<td>NEUT</td>
<td>Same</td>
<td>Same</td>
<td>3.15</td>
</tr>
<tr>
<td>LYMPH</td>
<td>&quot;</td>
<td>&quot;</td>
<td>2.43</td>
</tr>
<tr>
<td>MONO</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0.53</td>
</tr>
<tr>
<td>EO</td>
<td>&quot;</td>
<td>&quot;</td>
<td>1.13</td>
</tr>
<tr>
<td>BASO</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0.03</td>
</tr>
</tbody>
</table>
Table 6: Parameters shows the highest, lowest and mean value of pluckhers groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Highest</th>
<th>Lowest</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
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<td>2.99</td>
<td>7.37</td>
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<tr>
<td>RBC</td>
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<td>2.05</td>
<td>3.385</td>
</tr>
<tr>
<td>HGB</td>
<td>13.1</td>
<td>3.8</td>
<td>9.947</td>
</tr>
<tr>
<td>HCT</td>
<td>30.8</td>
<td>16.0</td>
<td>25.06</td>
</tr>
<tr>
<td>MCV</td>
<td>89.8</td>
<td>62.9</td>
<td>74.13</td>
</tr>
<tr>
<td>MCH</td>
<td>28.3</td>
<td>15.5</td>
<td>21.4</td>
</tr>
<tr>
<td>MCHC</td>
<td>31.7</td>
<td>23.8</td>
<td>28.76</td>
</tr>
<tr>
<td>PLT</td>
<td>291</td>
<td>17</td>
<td>167</td>
</tr>
<tr>
<td>RDW-SD</td>
<td>63.8</td>
<td>39.4</td>
<td>47.7</td>
</tr>
<tr>
<td>RDW-CV</td>
<td>24.9</td>
<td>13.9</td>
<td>19.05</td>
</tr>
<tr>
<td>PDW</td>
<td>Same</td>
<td>Same</td>
<td>22.1</td>
</tr>
<tr>
<td>MPV</td>
<td>&quot;</td>
<td>&quot;</td>
<td>14.0</td>
</tr>
<tr>
<td>P-LCR</td>
<td>&quot;</td>
<td>&quot;</td>
<td>53.0</td>
</tr>
<tr>
<td>PCT</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0.16</td>
</tr>
<tr>
<td>NEUT</td>
<td>4.83</td>
<td>1.31</td>
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<td>LYMPH</td>
<td>2.12</td>
<td>0.82</td>
<td>1.638</td>
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<td>0.31</td>
<td>0.576</td>
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<tr>
<td>EO</td>
<td>2.08</td>
<td>0.31</td>
<td>1.163</td>
</tr>
<tr>
<td>BASO</td>
<td>0.10</td>
<td>0.03</td>
<td>0.066</td>
</tr>
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</table>
DISCUSSION

Haematological parameters has been associated with their physiological state and may be induced directly by genotoxic compounds. The variation in parameters have been demonstrated to be sensitive to different toxic agents, they can be used for detecting pollutants or pesticides exposure in the environment (National Research Council, 1989). Pyrithroids pesticides are commonly used to control insects pest and represents about 30% (Amedur et al., 1991) of world insecticide consumption. Some authors (Reddy and Bashamohideen, 1989; Chauhan et al., 1994; Agarwal and Chaturvedie 1995; Nath, 1996) have reported a decreases in haematocrit haemoglobin and red blood cells values of some fish after their exposure to insecticides. Saxena and Seth (2002) showed a significant change in the haematology of the common fresh water fish. The information suggests that haematological parameters could be used as potential biomarkers of pyrethroid insecticides. Pyrethroids belong to a lipophilic insecticides class which are easily degraded in natural environments. A decrease in important blood parameters has been reported during exposure to various pesticides. The blood sample studies in the tea garden labour showed haemoglobin percentage very low. The result showed that 75% among factory workers, 80% among spraying group and 94% among pluckers found low. So, the workers in three groups showing haemoglobin percentage decreased significantly. This indicates that pesticides may
caused anaemia. This may be due to a decrease rate of production of red blood cells or an increased loss of these cells. Gill and Epple (1993) have attributed anaemia to impaired erythropoiesis due to a direct effect of pesticides or metal on hematopoietic centres (kidney/spleen). Accelerated erythroclasia due to altered membrane permeability and or increased mechanical fragility. Defective Fe metabolism or impaired intestinal uptake of Fe due to mucosal lesions.

Decrease in haemoglobin, haematocrit platelets counts, was observed the disturbed haemoglobin synthesis may be due to an effect of pesticides may result in anaemia. White blood cells plays a major role in the defence mechanism of the organisms and consists of granulocytes, monocytes, lymphocytes and thrombocytes. Granulocytes and monocytes function as phagocytes to salvage debris from injured tissue and lymphocytes produce antibodies (Ellis et al., 1978; Wedemeyer and Mcleay, 1981).

Blood parameters showed higher concentration of leucocytes. An increase in lymphocytes number may be the compensatory response of lymphoid tissues to the destruction of circulating lymphocytes (Shah and Altindag, 2005; Allen, 1994) observed increased WBC (lencocytes) counts in oreochromis aureus after mercury exposure. The increase in WBC observe din the present study could be attributed to a stimulation of the immune system in response to tissue damage caused by mercuric chloride. Gill and Pant (1985) have reported that the stimulation of the immune system causes
an increase in lymphocytes by an injury or tissue damage. Dhanekar et al. (1985) reported increase in large lymphocyte, reduction in small lymphocyte and thrombocyte populations as also elevation in monocyte, neutrophil and eosinophil cells in euteropneustes fossils.

The blood reflects pathophysiological status and its parameters are important in diagnosis of the structural and functional status of exposed to toxicant Sampath et al., 1998). Haematological findings reveals that exposure of pesticides may have toxic manifestations with evident effects. Increased production of erythropoietin may cause anaemia with chronic disease like infections and neoplastic conditions. Defective platelet functions is suspected in patients who show skin and mucosal haemorrhages. The result showed 33% in spraying group 25% in factory and 52% in pluckers platelets count below the normal range. These may be due to the exposure of pesticides. Reduction of platelets number caused various forms of thrombocytopenias and thrombolytosis occur due to risk in platelets count (Dacie and Lewis, 2006).

Eosinophil in plucking 94% and 75% in factory group found higher basophil 13% in spraying and 11% in plucking found higher. The increase number in eosinophils shoed eosinophilia. Eosinophilia can occur due to anaemia, malignant diseases with metastases etc. Basophil increase in the number showed basophilia. It may cause due to chloric myeloid leukaemia, action of chemicals or drugs etc. Basically environmental factors play a role
in the change of haematological parameters. Such as damaged due to exposure of radiation, benzene, tobacco smoking and exposure to agricultural chemicals like pesticide and associate with increase of rise of development of hematopoietic malignancies (Dacie and Lewis, 2006). An abnormal blood count indicate a primary haematological problems.

Across the globe pesticides have been found n human blood, urine, breast milk, semen, adipose tissues etc. Cumulative exposure to pesticides may come from food, water, air, dust soil etc. Pesticides can be absorbed through skin contract, inhalation or accidental ingestion. Farm workers come into direct contact with pesticides at work as well and occupationally exposed to them. When a person is exposed to pesticides, body’s detoxification mechanism re activated. Some pesticides metabolised into different chemicals and excreted and some are stored in fatty tissue in the body (Mathur et al., 2005).

Low mean corpuscular volume (microcytic) associated with low mean cell haemoglobin. It indicates that the tea garden labour exposed to pesticides can cause microcytica (Dacie and Lewis, 2006).

Decrease in the WBC count may suggest that some immunotoxic substance like certain pesticides can cause reduction in WBC counts in organisms exposed to this. Certain heavy metal (Dey et al., 1999) and pesticide name been reported to have echinocytogenic effects. DDT exposure in C. puncatus decrease in both haemoglobin content an the
number of enythrocytes leading to anaemia (Lone and Javaid, 1976). A study of surface damage followed by increased permeability of membrane leading to haemolysis was recorded in RBC of higher animals including human beings when treated with various classes of pesticides (Agrawal et al., 1990). However, a thorough ecotoxicological and ultrastructural studies involving different tissues in different animals are required for drawing a concrete conclusion. Health hazard due to the consumption of pesticides are various n the exposure of pesticides (Roy and Gupta, 2006).

Haematological attentions may be used for diagnosis in the field to asses pollution related pathophysiological alternations in human beings. The work may indicates that haematological parameters is a very useful diagnostic test for any kind of pesticide exposure for any organisms.

In conclusion results of the present investigation show that pesticides may cause immunological impairments in human beings. The pesticides exposure may damaged immune system and may result in severe physiological problems ultimately leading to the death of human being.

From the above findings the DLC gives the idea about the changes in blood due to exposure to the pesticide in the garden labourers. Reduction of WBC makes the person more susceptible to infections while monocyte and eosinophils will indicates the person to be affected by allergies. The platelet counts indicate that the exposure of pesticides may increase the risk of cardiovascular disease.
Mean cell haemoglobin concentration measure was used to assess the amount of red cell swelling (decreased MCHC) or shrinkage (increased MCHC) present (Millian and Wood, 1982). The measuring of haematological parameters which are used as valuable information. The employment of haematological techniques has provided valuable knowledge for any organisms in the assessment of health and in monitoring stress responses.

The measuring of haematological parameters, which are used in this study, has provided valuable information. The employment of haematological techniques has provided valuable knowledge for biologists in the assessment of health and in monitoring stress responses. We assume that variation in values of blood indices may be a defensive mechanism against toxicity through stimulation of erythropoiesis. We believe that further researches is needed to protect the organisms safe from pesticides.
PHOTOGRAPHS SHOW COLLECTION OF BLOOD SAMPLES FROM ROSEKANDY TEA GARDEN IN THREE DIFFERENT GROUPS (PLUCKERS, FIELD WORKERS & SPRAYERS) ALONG WITH EFFECT OF PESTICIDES ON THE BODY OF THE SPRAYERS