Chapter-4

Effect of lambda cyhalothrin on haematological parameters of rat, especially on alterations in morphology and oxidative stress in erythrocytes and the attenuating role of taurine

4.1. Introduction
4.2. Materials and methods
4.3. Results
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Abstract

Lambda cyhalothrin, a third generation type II pyrethroid, is used predominantly in agriculture production and animal husbandry. A study was conducted to investigate lambda cyhalothrin induced oxidative stress, morphological changes of erythrocytes and other hematological biomarkers in rat and its amelioration by taurine, 2-amino ethane-sulfonic acid, a β-amino acid. Rats were randomly divided into six groups and lambda-cyhalothrin was orally administered at two dose levels (10.83 and 15.17 mg/kg body wt), singly or combined with pretreated taurine (50 mg/kg body wt) for 14 consecutive days. Treatment of lambda cyhalothrin resulted in an increase in malondialdehyde, oxidized glutathione level and depletion of reduced glutathione level, superoxide dismutase, catalase, glutathione-s-transferase and glutathione peroxidase activity in erythrocyte compared to control. Scanning electron microscopic studies showed a marked alteration in the morphology of lambda cyhalothrin treated erythrocytes. Lambda cyhalothrin exposure also showed a significant decrease in erythrocyte count, haemoglobin percentage, haematocrit and red cell indices, whereas a significant increase in white blood cells and lymphocyte count were observed. However, pretreatment with taurine significantly restored the above said parameters. These findings revealed that lambda cyhalothrin exposure produced oxidative stress, morphological changes of erythrocytes and other hematological biomarkers and its amelioration was accomplished by taurine through its ROS scavenging activity.

Rini Ghosh, Ananya Pradhan, Pralay Maity, Kuladip Jana, Sujata Maiti Choudhury. Lipid peroxidative damage, alterations in antioxidant status and morphology in rat erythrocytes on lambda-cyhalothrin exposure and its attenuation by taurine. Toxicology and environmental health science-ACCEPTED
4.1. Introduction

Haematology symbolizes the study of the numbers and morphology of the cellular components of the blood, the red cells (erythrocytes), white cells (leucocytes), and the platelets (thrombocytes), a good indicators of the physiological status of animals (Khan and Zafar, 2005). Haematological studies are useful in the diagnosis of many diseases as well as the damage of blood cells, (Togun et al., 2007), also play a significant role in toxicological research (Siakpere et al., 2008). These are used to decide systemic relationship and physiological adaptations of animals and commonly measured variables. Many reports can be found regarding the pyrethroid-induced biochemical and physiological changes in target organs but not much attention has been paid to the effects of pesticides on non-target organisms. To understand the various status of the body and to determine stresses due to environmental, nutritional and/or pathological factors, haematological parameters are often used (Afolabi et al., 2011).

4.1.1. Haematological components and their functions

Haematological components consist of red blood cells that serve as a carrier of haemoglobin, white blood cells or leucocytes that fight against infections, platelets that are associated with blood clotting. Mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration are valuable in monitoring toxicity especially with feed constituents that affect blood as well as the health status of farm animals (Etim et al., 2014). Haemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates exception of
the fish family (Sidell and O’ Brien, 2006). The percentage (% of red blood cells in blood is known as packed cell volume (PCV) or haematocrit (Ht or Hct) or erythrocyte volume fraction (EVF) (Purves et al., 2003), involved in the transport of oxygen and absorbed nutrients.

![Diagrams of Cells](image)

**Figure 4.1.** Haematological components

**Source:** American society of oncology, 2004©

### 4.1.2. Haematological changes induced by pyrethroids in bird, fish and mammalian species

Pyrethroids are the major source of potential environmental hazards not only to birds, fish, and other animals but also to humans when they become part of food chains (Abd-Alla et al., 2002). Few workers reported non-significant changes in total erythrocyte count, haemoglobin percentage and haematocrite value in various animals treated with cypermethrin (Haratym-Maj, 2002; Sayim et al., 2005) whereas few studies reported that animals were suffering from anemia when treated with cypermethrin (Shah et al., 2007; Khan et al., 2009). In female mice, deltamethrin led to anemia which indicated downfall of erythropoiesis and haemoglobin synthesis.

### 4.1.3. Oxidant-antioxidant balance in erythrocyte
Oxidative stress is a condition that takes place by the disruption of oxidant-antioxidant balance which may lead to deleterious cellular damages (Ha et al., 2010). Antioxidants have protective role against free radicals and oxidative attack. By converting oxidants into non-toxic molecules it shields the organism from oxidative stress (Gate et al., 1999). It has been already confirmed that erythrocytes and their membranes are very much influenced by oxidative damage because of the presence of unsaturated fatty acids that are continuously exposed to high concentration of oxygen (Ney et al., 1990). Despite their well-equipped antioxidant defense system, erythrocytes may be oxidatively injured, because of exposure to toxic chemicals like pyrethroids.

4.1.4. Effects of lambda cyhalothrin on haematological system

Lambda cyhalothrin (LCT) appears to be a third generation type II pyrethroid, displays significant toxicological changes in rabbit peripheral blood lymphocytes (Georgieva, 2006), but there is no information regarding the LCT-induced morphological changes in mammalian erythrocytes.

4.1.5. Role of taurine in haematological system

Taurine, 2-aminoethanesulfonic acid is reported to find clinical application against a variety of pollutants where cellular damage is a result of reactive oxygen species (Issabeagloo et al., 2011). Through its cytoprotective, osmoregulatory, and membrane stabilization properties, taurine shows the ability to mitigate chlorpyrifos and lead induced hematotoxicity in the rats (Akande et al., 2014). The current study is designed to examine the lambda cyhalothrin induced damages inflicted on the rat
blood cells and to get to know the possible ameliorative role of taurine in these situations.

4.2. Materials and methods

4.2.1. Chemicals

Lambda cyhalothrin(LCT) 5% emulsifiable concentrate (EC) was procured from RPC Agro Industries, Kolkata. Taurine was purchased from Sigma Aldrich Inc., USA. Hydrogen peroxide(H$_2$O$_2$), Red blood cell dilution fluid, White blood cell dilution fluid, Drabkin’s diluents, Hydrochloric acid (HCl), Sulfo salicylic acid, Dithionitrobenzoic acid(DTNB), Tris-HCl, Pyrogallol, Thiobarbituric acid(TBA), n-Butanol-pyridine, Acetate buffer, Acetate buffer, Fructose, Ferric chloride(FeCl$_3$), Glacial acetic acid, Sodium chloride(NaCl), Phosphate buffer(PBS), Disodium hydrogen phosphate(Na$_2$HPO$_4$), Potassium di hydrogen phosphate (KH$_2$PO$_4$), Sodium di hydrogen phosphate( NaH$_2$PO$_4$), Sodium hydroxide(NaOH), Ethylenediaminetetraacetic acid(EDTA), n-Butanol-pyridine, Pentobarbital sodium, reduced glutathione(GSH), 1-Chloro-2,4-Dinitrochlorobenzene(CDNB), 2-vinylpyridine, Sodium azide, Histopaque-1077, Glutaraldehyde and other chemicals were purchased from Sigma-Aldrich, St. Louis, MO, USA; Himedia India, Ltd., Mumbai, India; and Merck India, Ltd., Mumbai, India for the experimentation.

4.2.2. Animal care

For the present study mature Wistar male albino rats (weighing 130-150 g) were taken and kept under controlled temperature(25 ± 2°C) and light conditions (12h-
light–dark cycle) with free access to water and standard laboratory feed throughout the period of experimentation i.e. 14 consecutive days. The rats were acclimatized for a week before the beginning of the experiments. All the animal experimental protocols were approved by the Institutional Animal Ethical Committee (IAEC), registered under Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India. All animal treatment and surgical procedures were carried out in accordance with the relevant laws and guidelines of the CPCSEA.

4.2.3. Treatment protocol

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
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<tbody>
<tr>
<td>Gr-I: Control</td>
<td>No treatment</td>
</tr>
<tr>
<td>Gr II: Taurine control</td>
<td>50mg/kg body wt.</td>
</tr>
<tr>
<td>Gr III: LCT low dose</td>
<td>1/7 of LD$_{50}$ value i.e., 10.83 mg/kg body wt.</td>
</tr>
<tr>
<td>Gr IV: Taurine + LCT low dose</td>
<td>50mg/kg body wt. + 10.83 mg/kg body wt</td>
</tr>
<tr>
<td>Gr V: LCT high dose</td>
<td>1/5 of LD$_{50}$ value i.e., 15.17mg/kg body wt.</td>
</tr>
<tr>
<td>Gr VI: Taurine + LCT high dose</td>
<td>50mg/kg body wt. + 15.17mg/kg body wt.</td>
</tr>
</tbody>
</table>

Total animals were divided into six groups (n=6). Final doses of LCT of the study for the present treatments were selected by preliminary investigations followed by oral LD$_{50}$dose 75.85mg/kg body wt. (Sharma et al.,2010). Taurine was applied at the dose level of 50 mg/kg body wt.(Ozden et al.,2009; Cetiner et al.,2005).

4.2.4. Sample collection
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After the last day of treatment, all the animals were sacrificed under light anesthesia using pentobarbital sodium and blood samples were collected from all the treated and control animals for the assessment of oxidative stress parameters, erythrocyte morphology and other haematological parameters.

4.2.5. Separation of erythrocytes

Using ethylene-diamine-tetra-acetic acid (EDTA) as an anticoagulant, 2 ml of blood was collected from the hepatic vein of each rat and was poured upon the same amount of histopaque-1077. Then it was centrifuged at 2000 rpm for 30 minutes to separate the erythrocytes. By removing plasma and leukocytes, only erythrocytes were suspended in phosphate buffer (0.1 M, pH 7.4) at 5% (v/v) concentration and were used for the assay of oxidative stress in rat erythrocytes.

4.2.6. Study on oxidative stress parameters in rat erythrocytes

4.2.6.1. Estimation of malondialdehyde (MDA)

MDA of erythrocyte suspension was determined according to modified method of Ohkawa et al. (Ohkawa et al., 1979) and the absorbance was taken at 535nm.

4.2.6.2. Estimation of reduced glutathione (GSH)

Reduced glutathione of erythrocytes was carried out according to the method of Griffith, (Griffith, 1981) and expressed as µg/mg Hb.

4.2.6.3. Estimation of oxidized glutathione (GSSG)

Oxidized glutathione was determined according to the method of Griffith, (Griffith, 1980). The absorbance was taken at 412 nm within 1min.
4.2.6.4. Superoxide dismutase (SOD) activity

SOD activity of erythrocytes was measured according to the method of Marklund and Marklund (Marklund and Marklund, 1974). Briefly, in a spectrophotometric cuvette, 2 ml of Tris-HCl (50 mM), 100 µl of 2 mM pyrogallol in the presence of 1 mM EDTA and 10 µl of erythrocyte suspensions were added and the reading was taken at 420 nm for 3 min. The enzyme activity was estimated by measuring the percentage inhibition of the pyrogallol autoxidation by SOD.

4.2.6.5. Assay of catalase (CAT) activity

Catalase activity was measured by adding 1 ml of 30 mM H₂O₂, 1.9 ml of 50 mM phosphate buffer and 0.1 ml of erythrocyte samples (in 0.05M Tris-HCl) in a glass cuvette. After mixing, six readings were noted at 240 nm at 30 sec interval (Aebi, 1974).

4.2.6.6. Evaluation of glutathione-s-transferase (GST) activity

Glutathione-s-transferase activity in erythrocytes was estimated spectrophotometrically according to the method of Habig et al. (Habig et al., 1974). Reaction mixture contained 0.1 ml of erythrocyte suspension, 2.8 ml of PBS, 0.1 ml of GSH and 50 µl of 60 mM CDNB. All the contents were taken in a cuvette and reading was noted at 340 nm. The values were expressed in µmol CDNB conjugate formed/min/ per milligram of Hb for erythrocytes.

4.2.6.7. Assay of glutathione peroxidase (GPₓ) activity

Determination of GPₓ was carried out by the method of Rotruck et al. (Rotruck et al., 1973). Briefly, the reaction mixture contained 0.2 ml of 0.4M Phosphate buffer
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(pH-7), 0.1ml of 10mM sodium azide, 0.2ml of erythrocytes suspension in phosphate buffer (pH-7), 0.2ml of 4mM reduced glutathione, and 0.1ml of 2.5mM hydrogen peroxide (H2O2). The contents were incubated for 10min at 37°C, and 0.4ml of 10% TCA was added to stop the reaction and centrifuged at 3200 rpm for 20min. Then 1 ml of 5,5'-dithiobisnitrobenzoic acid (DTNB) and 3 ml of di sodium hydrogen phosphate (Na2HPO4) were added to supernatant and the optical density was measured at 420 nm.

4.2.7. Scanning electron microscopic (SEM) study of rat erythrocytes

Scanning electron microscopic study (Goel et al., 2006) of rat erythrocytes were done by taking blood sample from each animal. Erythrocytes were separated using Histopaque-1077 and then 500µl of erythrocytes were immediately fixed in 2.5% glutaraldehyde made in 0.1 M phosphate buffer (pH 7.4). After 1 h of fixation, cells were centrifuged at 1000–1500 rpm and pellets were suspended in triple distilled water. After single washing, the final pellet was again suspended in triple distilled water. A drop of the sample was smeared on the metallic SEM stubs, loaded with a conductive silver tape on its top. The stubs were then coated with gold to a thickness of 100 Å using a sputter-ion coater, for 4–5 min and the specimens were finally ready to observe under scanning electron microscope.

4.2.8. Total erythrocyte count (Red Blood Cell count)

Total erythrocyte count (Wintrobe, 1967) was done by diluting blood in 1:200 dilutions with RBC dilution fluid and then total erythrocytes were counted in Neubaur haemocytometer chamber and were expressed as ×10^6 mm^-3.
4.2.9. Estimation of haemoglobin percentage

The haemoglobin percentage was measured by cyanmethemoglobin method (Dacie and Lewis 1975).

4.2.10. Packed cell volume (PCV)

Packed cell volume (PCV) was measured by taking anticoagulated whole blood, which was centrifuged at 3000 rpm for 30 minutes in a Wintrobe’s tube. The erythrocytes were settled down at the bottom (Wintrobe, 1967). The PCV was determined by the height of erythrocyte column which is directly read from the graduation mark on the Wintrobe’s tube.

4.2.11. Red cell indices

Red cell indices, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were measured from the total erythrocyte count, haemoglobin percentage and haematocrit value (Wintrobe, 1967).

I. Mean corpuscular volume (MCV): The average volume of erythrocyte is known as mean corpuscular volume and is expressed in ‘Fentolitres’.

\[
MCV = \frac{\text{Haematocrit (\%)} \times 100}{\text{RBC count in million}}
\]

II. Mean corpuscular haemoglobin (MCH): The average weight of haemoglobin content in erythrocyte is called mean corpuscular haemoglobin and is expressed in ‘Picograms’.
III. Mean corpuscular haemoglobin concentration (MCHC): Expression of the average haemoglobin concentration per unit volume of packed red cell is defining as mean corpuscular haemoglobin concentration and is expressed in ‘%’.

\[ \text{MCHC} = \frac{\text{MCH}}{\text{MCV}} \times 100 \]

4.2.12. Total leukocyte count (TLC)

Total leukocyte count (Wintrobe, 1967) was done by diluting blood in 1:20 dilution with white blood corpusal (WBC) dilution fluid and then total leukocytes were counted in Neubaur haemocytometer chamber.

4.2.13. Differential leukocyte count (DLC)

Thin blood smear was made by anticoagulant-added whole blood in a clean glass slide and was stained with Leishman’s stain and then was observed under oil immersion objective of the microscope. An area of the blood smears slightly before than `tail end` was chosen where the morphology of the white cells is clearly visible. The percentage of granulocytes and agranulocytes were calculated (Wintrobe, 1967).

4.2.14. Statistical analysis

The data were analyzed to achieve mean values and standard errors for all treated and control samples. Statistical analyses of the collected data were completed by a one-way analysis of variance (ANOVA), followed multiple comparison two tail t-
test for analysis between groups, using Origin 6.1 software. Results were presented as mean ± SEM. Difference was considered statistically significant when p< 0.05.

4.3. Results

4.3.1. Alterations in oxidative stress parameters

The results of the present study showed that MDA level was significantly increased (p<0.001) in erythrocytes of the LCT treated groups compared to the control where MDA level decreased significantly (p<0.001) in taurine + LCT-treated groups (fig-4.2.A).

The GSH levels in erythrocytes of high dose LCT-treated rat were altered significantly (p<0.001) compared to control (fig-4.2.B). Administration of taurine to the low and high dose LCT-treated animals resulted in restoration of erythrocyte GSH levels significantly (p<0.05). A significant increase (p<0.001) in GSSG level in high dose LCT treated rat erythrocytes was seen in figure-4.1.C. It was significantly (p<0.001) reduced after pretreatment of taurine.

In figure 4.2.D the lambda cyhalothrin caused a significant decrease (p<0.01) in the activity of SOD in treated group compared to the control. Pretreatment of taurine along with lambda cyhalothrin improved the parameters significantly (p<0.05). Activity of CAT was diminished significantly (p<0.001) in LCT treated group (Fig-4.2.E). Taurine supplementation along with lambda cyhalothrin caused increase CAT activity significantly (p<0.001).
Significantly decreased in the activity of GST and GP\textsubscript{X} were also observed in the LCT-intoxicated rats (Fig-4.2.F, 4.2. G). Pretreatment of taurine was found to exhibit a protective effect on the GST and GP\textsubscript{X} activity.
Figure-4.2. Effect of lambda cyhalothrin and taurine on lipid peroxidation (MDA) and antioxidant status in control and experimental group of rat erythrocytes. Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a, Group I versus all other groups; superscript b, Group III versus Group IV and superscript c, Group V versus Group VI (*indicates p<0.05, ** indicates p<0.01, *** indicates p<0.001). A= Effect of lambda cyhalothrin and taurine on MDA, B= Effect of lambda cyhalothrin and taurine on GSH, C= Effect of lambda cyhalothrin and taurine on GSSG, D=Effect of lambda cyhalothrin and taurine on SOD, E=Effect of lambda cyhalothrin and taurine on CAT, F=Effect of lambda cyhalothrin and taurine on GST, G=Effect of lambda cyhalothrin and taurine on GPx.

4.3.2. Scanning electron microscopic observations of rat erythrocyte morphology.

Effects of LCT intoxication along with pretreatment of taurine on rat erythrocytes were observed under scanning electron microscope and the results are depicted in figure 4.3.

Normal control animals showed perfect discocytes (fig 4.3.Gr-I). No significant morphological abnormalities in blood cells were observed in the animals treated with taurine alone (fig 4.3. Gr-II). However, animals treated with LCT in low doses showed morphological alterations from discocytes (D) to stomatocytes (S) and leptocytes (fig 4.3. Gr-III). The red cells with stoma in middle part of the cells called stomatocytes along with folded bowl shaped. Few ovalocytes(O) were observed in high dose LCT treated animals (fig 4.3.Gr-V). Certain irregularly created and
contracted cells with numerous projections known as echinocytes (E) were also visible in the LCT treated high dose group animals (fig 4.3, Gr-V). Dacrocytes (T) i.e. tear drop like structure were also found in this LCT treated high dose group (fig 4.3, Gr-V). Protective effects of taurine were evident after the pretreatment of taurine followed by LCT, where the drastic alterations in the shape of the blood cells were restored close to the normal appearance of the cells (fig 4.3, Gr-IV and Gr-VI). Despite all these protective effects of taurine, very low population of echinocytes were still present in control and taurine control group (fig-4.3, Gr-I and Gr-II).

4.3.3. Effects on haemogram

Figure 4.3. Scanning electron photomicrograph of erythrocytes of control and treated rats. Bar scale = 10 µm. Discocytes (D), Leptocyte (L), stomatocyte (S), ovalocyte (O), echinocyte (E), dacrocyte or tear drop like structure (T). Bar scale = 10 µm.
In LCT treated groups total erythrocyte count and haemoglobin (%) were decreased significantly (p<0.001) at dose-dependent manner (Table 4.1) which demonstrated the toxic effects of LCT. Pretreatment of taurine reduced the toxic effects of LCT and caused significant increase in total erythrocyte count and haemoglobin percentage. PCV, MCH and MCHC were decreased significantly (p<0.001) in LCT treated group compared to control group (Table 4.1.) whereas MCV value was increased significantly in case of LCT-intoxicated group (Table 4.1). Taurine restored these parameters towards more or less control level (Table 4.1).
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Table 4.1. Effect of lambda cyhalothrin and taurine on total erythrocyte (RBC) count, Haemoglobin concentration (Hb conc.), PCV (Packed cell volume), Mean cell volume (MCV), Mean cell haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) in control and experimental group of rat.

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<tr>
<td>Total Erythrocyte Count (×10^6/mm^3)</td>
<td>7.3±0.09</td>
<td>7.5±0.15</td>
<td>6.3±0.12</td>
<td>7±0.1</td>
<td>5.8±0.18</td>
<td>6.05±0.1</td>
</tr>
<tr>
<td>Hb percentage (gm/dl)</td>
<td>14.73±0.03</td>
<td>14.7±0.05</td>
<td>11.86±0.04</td>
<td>13.4±0.07</td>
<td>10.31±0.04</td>
<td>11.5±0.15</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>44±0.14</td>
<td>44.8±0.15</td>
<td>43.68±0.07</td>
<td>44.48±0.22</td>
<td>41.05±0.31</td>
<td>42±0.17</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>60.27±0.26</td>
<td>59.73±0.2</td>
<td>67.62±0.08</td>
<td>61.43±0.21</td>
<td>70.69±0.02</td>
<td>68.86±0.23</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>20.18±0.02</td>
<td>19.6±0.09</td>
<td>18.82±0.03</td>
<td>19.14±0.05</td>
<td>17.78±0.06</td>
<td>18.85±0.03</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>33.48±0.05</td>
<td>32.81±0.58</td>
<td>27.8±0.58</td>
<td>31.16±0.04</td>
<td>25.14±0.57</td>
<td>27.37±0.79</td>
</tr>
</tbody>
</table>

Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a Group I versus all other groups; superscript b Group III versus Group IV and superscript c Group V versus Group VI (*indicates p<0.05, ** indicates p<0.01 and *** represents p<0.001).
4.3.4. Effects on leukogram

Leukocyte count and lymphocyte percentage (Table 4.2) were increased significantly (p<0.001) in LCT treated groups. The results showed that the treatment of taurine to the rats exhibited significant decrease in leukocyte count and lymphocyte percentage compared to rats receiving only LCT. Neutrophil count also decreased significantly (p<0.001) at dose-dependent manner in case of LCT treated rats. In LCT treated low and high dose groups, eosinophil and monocyte percentage were decreased significantly (p<0.001) also in a dose-dependent manner compared to that of control.
Table 4.2. Effect of lambda cyhalothrin and taurine on total leukocyte count, lymphocyte count, neutrophil count, eosinophil count, and monocyte count in control and experimental group of rat.

<table>
<thead>
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<tbody>
<tr>
<td>Total leukocyte count (×10^6/µl)</td>
<td>6.6±0.12</td>
<td>6.6±0.06</td>
<td>8.4±0.05*</td>
<td>7.9±0.02a***</td>
<td>9±0.23a***</td>
<td>8.3±0.1a*<strong>c</strong></td>
</tr>
<tr>
<td>Lymphocyte count (%)</td>
<td>56.13±0.13</td>
<td>56.05±0.13</td>
<td>60.15±0.26a***</td>
<td>58.0±0.16a**<em>b</em></td>
<td>65.83±0.17a***</td>
<td>62.05±0.26a<em><strong>c</strong></em></td>
</tr>
<tr>
<td>Neutrophil count (%)</td>
<td>36.9±0.57</td>
<td>37.15±0.83</td>
<td>34.0±0.54a*</td>
<td>35.7±0.28</td>
<td>29.97±0.5a***</td>
<td>32.05±0.25a***</td>
</tr>
<tr>
<td>Eosinophil count (%)</td>
<td>2.5±0.06</td>
<td>2.6±0.09</td>
<td>2±0.1a**</td>
<td>2.4±0.12b*</td>
<td>1.4±0.06a***</td>
<td>2±0.10a<em><strong>c</strong></em></td>
</tr>
<tr>
<td>Monocyte count (%)</td>
<td>4.4±0.19</td>
<td>4.2±0.14</td>
<td>3.45±0.16a**</td>
<td>3.9±0.12a<em>b</em></td>
<td>2.8±0.10a***</td>
<td>3.0±0.22a***</td>
</tr>
</tbody>
</table>

Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a Group I versus all other groups; superscript b Group III versus Group IV and superscript c Group V versus Group VI (*indicates p<0.05, ** indicates p<0.01 and *** represents p<0.001).
4.4. Discussion

In the study lambda cyhalothrin intoxication created alterations in oxidative stress parameters in rat erythrocytes as well as produced a notable variation in the morphological appearance of the rat erythrocytes and remarkable changes in the haematological parameters. Protective effect of taurine was also shown in the results. The erythrocytes in normal physiological situations are resistant to oxidative damage due to their well-equipped biological and protective mechanisms by enhanced antioxidant enzymes, such as catalase (CAT), Glutathione peroxidase (GPₓ), superoxide dismutase (SOD) and glutathione reductase (GR) (Sies, 1997). It must also be mentioned that the erythrocytes may be susceptible to oxidative damage because of the presence of heme-iron, PUFA and oxygen, under oxidative stress and it may trigger the reactions that make oxidative changes in red blood cells (Clemens and Waller, 1997). It has been reported that pyrethroids prompt oxidative stress, as displayed by elevation of lipid peroxidation products (Banerjee et al., 1999). LCT is an α-cyano moiety comprising type-II pyrethroid which instigates the oxidative stress. Nasuti et al. and Prasanthi et al. noticed that oxidative damage was done in erythrocytes because of lipophilicity of pyrethroids (Nasuti et al., 2003; Prasanthi et al., 2005). Elevated malondialdehyde (MDA) level in erythrocytes treated with LCT is an agreement with the findings of Nasuti and Prasanthi (Prasanthi et al., 2005). The pretreatment of taurine in conjunction with lambda cyhalothrin reduced the increased level of MDA towards its normal limit. The normalization of MDA level in erythrocytes following taurine pretreatment is very likely because its antioxidant
properties, as has been shown previously (Cabre et al., 1999). Reduced glutathione (GSH) is considered as a notable biomolecule that serves against chemically created oxidative stress. It acts as a free radical scavenger and neutralizes the radicals that are involved in biological damage (Nicotera and Orrenius, 1986). Hence, the measurement of its activity is necessary to evaluate the oxidative stress and antioxidant status created by LCT. In the current study, GSH content reduced notably in the erythrocytes of LCT treated rat compared to the control. Noteworthy deterioration in the GSH level by LCT exposure is either because of increased utilization or reduced production of GSH. Taurine pretreatment may lead to an enhancement in GSH levels by directing cysteine into the GSH synthesis as cysteine is the precursor of GSH (Hagar, 2004). Elevated oxidized glutathione (GSSG) level in case of LCT-treated erythrocytes and reduced GSSG in taurine treated erythrocytes also substantiate the earlier findings. This study shows that the reduction in the activity of superoxide dismutase, a copper-zinc-containing enzyme and CAT, a heam-containing enzyme in lambda-cyhalothrin intoxicated erythrocytes can be elucidated by the increase of lipid peroxidation followed by increase in MDA content. LCT created stress is, perhaps, the reason for over production of ROS that increases singlet oxygen and peroxyl radicals, which reduce SOD and CAT by their effective use. Glutathione peroxidase, a selenium comprising tetrameric glycoprotein found in mammalian erythrocytes helps to stop lipid peroxidation of the cell membrane (Brigelius-Flohé, 1999). Lambda cyhalothrin exposure causes a reduction in the activity of GPx, perhaps, because of the depleted GSH, as the activity of GPx relies upon the level of GSH. Increased use of GPx to detoxify the pesticide induced
free radicals, also substantiate the above result (Hayes and Pulford, 1995). Glutathione-s-transferase are detoxifying enzymes that catalyze the conjugation of a variety of electrophilic substrates to the thiol group of glutathione, releasing less toxic forms (Hayes and Pulford, 1995). The notable decrease of GST activity in erythrocytes after exposure of lambda cyhalothrin may suggest enough detoxification of pesticide in rat erythrocytes. Taurine administration reversed all the abnormalities because of its stimulatory effect on endogenous antioxidants (Saad and Al-Rikabi, 2002). This study tells us that the radical alterations in the red cell morphology of the animals exposed to LCT. The normal presence of the erythrocytes was changed into many different forms including echinocytes, stomatocytes, dacrocyes and few spherocytes also. Echinocyte form is thought to be a structurally pathological membrane defect, happening also during the smear preparation. LCT may disturb the structure of lipids situated in the erythrocyte membrane (Suwalsky et al., 2004). This means that erythrocytes in individuals exposed to LCT poisoning shall not survive their whole life span of 120-130 days, but are supposed to be removed as echinocytes. This leads to low hemoglobin levels because of LCT toxicity (Shashi and Meenakshi, 2012). Stomatocyte has the stoma in the middle part, normally found in liver disease, also noticed in LCT treated animals. Because of the lack of related studies in this field, the acceptable elucidations for such findings are the abnormal erythropoiesis, or the defects on the erythrocyte membrane lipid bilayer. Perhaps, it is because of insufficient haemoglobin formation, decreased water permeability across erythrocyte membranes, increased erythrocyte aging, the
rate of oxygen release by erythrocytes, reduced thermo stability of erythrocytes, or augmented erythropoiesis to compensate anemia (Tkeshelashvili et al., 1989).

Pretreatment of taurine followed by LCT enhanced the morphology of the red blood cells. The preventive effects of taurine are most likely because of its function as a direct antioxidant by scavenging reactive oxygen radicals, inhibition of lipid peroxidation and as an indirect antioxidant by averting changes in erythrocyte membrane permeability resulting from oxidative damage in many tissues including erythrocytes (Waters et al., 2001).

A notable change in haemogram, leukogram and red cell indices were noticed in rats exposure to lambda cyhalothrin suggests the physiological dysfunction of the haemopoietic system of rats. The decline in erythrocyte counts noticed with LCT treatment may be because of haemolysis as a result of type-II pyrethroid exposure which leads to haemorrhage and reduced erythropoiesis (Mandal et al., 1986). Substantial decline in erythrocyte count and haemoglobin percentage could perhaps be due to suppression of erythropoiesis and haem synthesis, and also due to destruction of erythrocyte in hemopoietic tissue (Fetoui et al., 2008). Lysis of erythrocyte is created by chemicals that cause harm to erythrocyte membrane and it leads to oxidative injury to haemoglobin, or may damage the anti-oxidative protective mechanism. Enhanced hemolysis generally causes decline in haemoglobin, erythrocyte count and are accompanied by high reticulocytes count, enhanced anisocytosis, increased red cell distribution width and volumes. Few authors have documented similar results with the treatment of cypermethrin in rats (Manna et al., 2004). Haemoglobin percentage and hematocrit values have direct
connection to erythrocyte count (Moustafa et al., 2012) due to the synergistic link among these blood parameters in all vertebrates. Reduction in haemoglobin percentage in the current study could be because of the reduced biosynthesis of haem in bone marrow enhanced rate of destruction of erythrocytes or declining rate of erythrocytes formation.

The packed cell volume(PCV) suggests oxygen carrying capacity of blood and the degree of stress on animal health. In high dose lambda cyhalothrin treated group PCV level declined. In agreement with the current result reduced erythrocyte count, haemoglobin percentage and PCV levels were also documented in rats treated with deltamethrin (Yekeen et al., 2007).

In our study mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were reduced in lambda cyhalothrin treated groups, which is, perhaps, suggestive of macrocytic and hypochromic anaemia (Barger, 2003). Because of lower erythrocyte count, haemoglobin percentage and PCV in LCT treated animals, MCH and MCHC value were diminished. In the current study, the rise in mean corpuscular volume (MCV) and decline in MCHC also suggests the probability of macrocytic and hypochromic anaemia (Barger, 2003; Latimer et al., 2004) perhaps, because of the increased activity of bone marrow and deficiency of some hemopoietic factors. Increased MCV may also be noticed in regenerative anaemia because of haemolysis and haemorrhages.

The rise in leukocyte was also reported in lambda cyhalothrin intoxicated groups, possibly due to the activation of immune system of the body (Yousef et al., 2003).
This may lead to an increase in release of leukocytes from bone marrow storage pool into the blood. The principle function of leukocytes is to safeguard against foreign bodies, which is achieved by leukocytosis and antibody production. Pathological leukocytosis, may be formed because of the exposure of chemicals or acute haemorrhages and haemolysis. Leukocytosis may be increased because of the resistance of the animal for localization of the inflammatory response. Another probable reason of leukocytosis is, the severe haemorrhages in liver and lungs (Latimer et al., 2004). This rise is, perhaps, connected to a rise in lymphocyte percentage.

However, pretreatment with taurine has a strong protective effect against lambda cyhalothrin created toxicity in haematological parameters of rats. The mechanism underlying haemato-protection of taurine is, perhaps, connected to its anti-anemic qualities which effectively supports to hemopoiesis. A number of investigators noted that taurine safeguards several organs in the body against toxicity and oxidative stress because of exposure of heavy metals and other toxins as well as drugs (Manna et al., 2009; Parildar et al., 2008).

4.5. Conclusion

In conclusion, it may be said that the lambda cyhalothrin treatment creates toxicity by producing oxidative stress and structural changes in erythrocytes and by alteration of haematological parameters in rat. Collectively, these data suggest that taurine pretreatment can potentially be considered as an intervention in human subject
against haematological dysfunction with accidental exposure of lambda cyhalothrin and related pyrethroids.

Figure 4.4 shows the schematic diagram of LCT induced haematotoxicity.
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4.6. References


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