INDUCED TOXIC POTENTIALS OF LAMBDA CYHALOTHRIN, A SYNTHETIC PYRETHROID ON HAEMATOLOGICAL PROFILES IN ALBINO MICE

*M. Suseela, K. Gokul, K. Jayantharao and P. Jacob doss
1Department of Zoology, Vikrama Simhapuri University, Nellore
*Author for Correspondence

ABSTRACT
The aim of the study was to know the haematological changes in albino mice following the treatment with Lambda cyhalothrin. In this study, mice were divided randomly into 4 equal groups A, B, C and D. Lambda cyhalothrin was dissolved in corn oil and administered to the mice orally at the dose rate of 24 mg/kg body weight with an interval of 48hrs for 10, 20, and 30 days. The animals were sacrificed on 11th, 21st and 31st day of the experiment and blood was collected for estimation of haematological profiles. λ-Cyhalothrin significantly (P<0.05) decreased in RBC counts, Hb concentration, PCV, MCV, MCH, MCHC and increased in WBC count. The study demonstrated the effect of Lambda cyhalothrin on haematological profiles.

Keywords: Lambda Cyhalothrin, Synthetic Pyrethroid, Haematological Profiles, Corn Oil

INTRODUCTION
The current trend of using excessive amount of insecticides and chemical fertilizers for increasing the productivity of crops to feed explosively growing populations. During this course some of the synthesized chemicals not only helped the man kind but also became reasons for his agony. A good number of chemicals in form of pesticides reigned for quite some time, however left many problems that were related to welfare of human beings (Sharma et al., 2010). The use of pyrethroid insecticides is increasing for agriculture, commercial pest control, and residential consumer use. In addition, there is a trend toward the use of newer and more potent compounds (Amweg et al., 2005). Synthetic pyrethroid insecticides are widely used in protection of fruits and vegetables as well as in the public hygiene due to their strong neurotoxic activity against insects (Tyrkiel et al., 2001). Lambda cyhalothrin (LCT) is a potent synthetic type II pyrethroid used worldwide to control a wide range of insects in agriculture, forestry, human health and home (Rachid et al., 2010), in agriculture, home pest control, protection of food stuff and disease vector control (Fetoui et al., 2009).

LTC has been found to cause adverse effects on many tissues, chromosomal aberrations and micronucleus formation in mouse bone marrow (Celik et al., 2003; Celika, 2005). However, their pathological effects have been encountered in experimental studies in different animals (Manna et al., 2004; Khan et al., 2003). Though pyrethroids formulated insecticide has been reported to be safe following normal haematological parameters seen in experimental studies with minimal exposure (Saka et al., 2011), some studies have reported its toxic effects (Inayat et al., 2007; Sangha et al., 2011) such as neurotoxicity, haematotoxicity and hepato toxicity (Sayim et al., 2005; Altug et al., 2006; Saxena and Saxena, 2010).

Blood findings are important for the assessment of various systemic functions and health of animals under various environmental conditions and most importantly for diagnosis of drug or chemical induced haemolysis (Atamanalap and Yanik, 2003). Therefore the present study has been designed to investigate the alterations in haematological parameters during sub acute toxicity of Lambda cyhalothrin.

MATERIALS AND METHODS
Chemical substances
Lambda-cyhalothrin is a synthetic pyrethroid insecticide (C23H19 ClF3NO3). CAS chemical name [a-cyano-3-phenoxybenzyl-3-(2-chloro-3, 3, 3-trifluoro-1-propenyl)-2,2-dimethylcyclo-propanecarboxylate]. CAS registry number 91465-08-6.
Preparation of test concentration
It was diluted in Corn oil for the final test concentration. The acute oral LD50 for Lambda cyhalothrin in albino mice was 24 mg/kg body weight. Therefore 4.8 mg/kg body weight (1/10th) as a dose was selected in the study.

Animals and treatment
Twenty four albino mice of initial body weight of 30-35g., were obtained from the animal house of Science Faculty, S.V University, Tirupathi. All animals were acclimatized for 10 days before the start of the experimental procedure. After 10 days of acclimation, the animals were randomly assigned to both the experimental groups and the control group, each containing 6 rats. The animals were housed in labeled cages with solid plastic sides and stainless-steel grid tops and floors, in a room designed for control of temperature (approximately 21±1°C), humidity (45-75%) and light cycle (12 h light, 12 h dark). Animals were orally fed daily a normal diet in standard laboratory pellets (10 g/day/rat). The first group (control group) of animals received the corn oil only and the other groups received Lambda cyhalothrin dissolved in corn oil at doses of 4.8 mg kg body weight by Gavage. 0.2 ml of solution was administered daily for 10, 20 and 30 days.

Blood collection
The blood was drawn from the orbital venous plexus by puncturing with the tip of Pasteur pipette under diethyl ether anesthesia and the blood was allowed to fall drop by drop into a graduated centrifuge tubes containing EDTA, anticoagulant to the required quantity for haematological work. The bleeding was arrested by gently pressing the eyeball with the help of dry cotton.

Hematological analysis
The blood sample with EDTA was used for hematological analysis. Blood parameters namely Red Blood Cell (RBC) counts, White Blood Cell (WBC) counts were determined by Davidson and Henry method, Hemoglobin (Hb) by Sahli’s method, Packed Cell Volume (PCV) by Schalm et al., method, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were determined by using standard reference methods of Benjamin.

Statistical analysis
Analyses were done using the SPSS software. The results of haematological analysis were presented as the mean ± SD. Comparisons were made between control and treatment groups using one-way Analysis of Variance (ANOVA) together with Dunnet’s tests. Values of p ≤ 0.05 were regarded as statistically significant.

RESULTS AND DISCUSSION

Figure 1: Changes in Haematological parameters in Albino mice exposed to Lambda Cyhalothrin
The changes in haematology of Lambda cyhalothrin treated albino mice are indicated in Table 1 and Figures. Oral administration of Lambda cyhalothrin produced a statistically significant decrease in RBC, Hb, PCV but WBC count shows increased level in group I, group II, and group III respectively. The red cell indicators like Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) are dependent on the RBC count, Hb concentration and PCV values. MCV, MCH and MCHC showed statistically significant decrease in experimental groups of Lambda cyhalothrin intoxication in albino mice.

Table 1: Effect of Lambda Cyhalothrin exposure on Red blood cells (RBC), Haemoglobin (Hb), Packed cell volume (PCV) and White blood cells (WBC) in albino mice

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Control</th>
<th>Group I (10 Days)</th>
<th>Group II (20 Days)</th>
<th>Group III (30 Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC Mean</td>
<td>8.640</td>
<td>6.854</td>
<td>5.183</td>
<td>4.486</td>
</tr>
<tr>
<td>SD ±0.200</td>
<td>±0.234</td>
<td>±0.196</td>
<td>±0.261</td>
<td></td>
</tr>
<tr>
<td>PC (-20.671)</td>
<td>(-40.012)</td>
<td>(-48.079)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb (gm/100ml) Mean</td>
<td>15.634</td>
<td>10.897</td>
<td>7.936</td>
<td>5.987</td>
</tr>
<tr>
<td>SD ±0.097</td>
<td>±0.042</td>
<td>±0.037</td>
<td>±0.010</td>
<td></td>
</tr>
<tr>
<td>PC (-30.299)</td>
<td>(-49.239)</td>
<td>(-61.705)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCV (%) Mean</td>
<td>43.924</td>
<td>32.840</td>
<td>24.342</td>
<td>20.586</td>
</tr>
<tr>
<td>SD ±0.033</td>
<td>±0.239</td>
<td>±0.044</td>
<td>±0.011</td>
<td></td>
</tr>
<tr>
<td>PC (-25.234)</td>
<td>(-44.582)</td>
<td>(-53.133)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC (cu.mm) Mean</td>
<td>12056.566</td>
<td>13284.018</td>
<td>14683.332</td>
<td>15989.723</td>
</tr>
<tr>
<td>SD ±472.996</td>
<td>±371.252</td>
<td>±364.687</td>
<td>±405.926</td>
<td></td>
</tr>
<tr>
<td>PC (10.181)</td>
<td>(21.787)</td>
<td>(32.623)</td>
<td></td>
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</tr>
</tbody>
</table>

The data in Table 1 show that there was a gradual decrease in erythrocyte count, haemoglobin content and the number of blood platelets in mice treated with Lambda cyhalothrin. These results are in agreement with those obtained from previous studies of the haematological effects of pyrethroids on mammalian animals.

Table 2: Effect of Lambda Cyhalothrin on Red cell indices

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Control</th>
<th>Group I (10 Days)</th>
<th>Group II (20 Days)</th>
<th>Group III (30 Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCV (µg) Mean</td>
<td>50.859</td>
<td>47.444</td>
<td>45.986</td>
<td>42.027</td>
</tr>
<tr>
<td>SD ±1.162</td>
<td>±0.373</td>
<td>±0.451</td>
<td>±0.322</td>
<td></td>
</tr>
<tr>
<td>PC (-6.715)</td>
<td>(-9.582)</td>
<td>(-17.366)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCH (pg) Mean</td>
<td>18.103</td>
<td>15.898</td>
<td>15.282</td>
<td>12.213</td>
</tr>
<tr>
<td>SD ±0.418</td>
<td>±0.159</td>
<td>±0.476</td>
<td>±0.079</td>
<td></td>
</tr>
<tr>
<td>PC (-12.180)</td>
<td>(-15.583)</td>
<td>(-32.536)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCHC (%) Mean</td>
<td>35.593</td>
<td>33.552</td>
<td>32.583</td>
<td>29.083</td>
</tr>
<tr>
<td>SD ±0.211</td>
<td>±0.282</td>
<td>±0.164</td>
<td>±0.045</td>
<td></td>
</tr>
<tr>
<td>PC (-5.733)</td>
<td>(-8.455)</td>
<td>(-18.289)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration

In the present investigation the toxic effect of Lambda cyhalothrin on the haemogram is determined in albino mice. Mice treated with Lambda cyhalothrin became anaemic and haematological analysis,
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revealed a reduction in Red Blood Cells (RBC) count, Haemoglobin (Hb) and in Packed Cell Volume (PCV); while the Leukocyte (WBC) count was increased in comparison to control animals. Collectively, the decrease in the erythrocyte count and haemoglobin content recorded in the present work indicated that Lambda cyhalothrin-treated mice were anaemic. Rachid et al., (2010) reported that alterations in haematological parameters were brought about by Lambda cyhalothrin as an anaemic condition because of decreased synthesis of RBC. The decrease in RBC counts observed with Lambda cyhalothrin treatment could be due to haemolysis as a result of type II pyrethroid which causes haemorrhages and reduced erythropoiesis (Mandal and Lahiri, 1989). One of the molecular mechanisms of toxicity of some pesticides seems to be lipid peroxidation; as a consequence these compounds can disturb the biochemical and physiological functions of the RBC (Akhgari et al., 2005). Reduction in Hb content could be due to the impaired biosynthesis of haem in bone marrow, increased rate of destruction or reduction rate in rate of formation of RBC’s. The decrease in RBC and Hb content could also be due to disruptive action of the pesticides on the erythropoietic tissue as a result of which the viability of the cells might be affected. Fetoui et al., (2008) also reported a decrease in some haematological parameters (RBC, Hb, Ht: p<0.01) in erythrocytes. In general anaemia, reduction in the number of red blood cells or of haemoglobin in the blood can reflect impaired synthesis of haemoglobin (eg, in iron deficiency) or impaired production of erythrocytes (eg, in folic acid or vitamin B12 deficiency) (Murray et al., 2007). Lambda cyhalothrin caused decrease in RBC, Hb and Hct, which might be due to the effect of pesticide on blood-forming organs suggesting the anaemic condition of the treated animals (Rachid et al., 2010; Fetoui et al., 2008). The results in the present investigation are in line with those found by Celika et al., (2005); Celika (2003) in bone marrow. Celika et al., (2005); Celika (2003) reported that Lambda cyhalothrin caused a significant decrease in number of polychromatic erythrocytes compared with controls. These observations indicate the invivo susceptibility of mammals to the genetic toxicity and cytotoxicity potential of Lambda cyhalothrin.

Anaemia, defined clinically as a decrease in haematocrit or Hb concentration, may be caused by blood loss, excessive haemolysis, and/or deficient erythropoiesis (Baynes and Dominiczak, 2005). In internal haemorrhages, some erythrocytes are absorbed by lymphatic vessels (autotransfusion) particularly in haemorrhages in body cavities. Remaining RBC's are lysed or phagocytosed (Latimer et al., 2004). Various authors reported similar results with the treatment of pyrethroids in rats (Manna et al., 2004; Ferah Sayim et al., 2005), Sheep (Yousef et al., 1998), rabbits (Yousef et al., 2003; Basir, 2005; Shah et al., 2007; Ahmad et al., 2011) and goats (Faridi, 2005), goats with molybdenum (Kusum et al., 2010). Amoudi, (2012), noted that there was a gradual decrease in erythrocyte count, haemoglobin content and the no. of platelets in mice treated with metalaxyl fungicide. Mokhtar, (2010) reported that aluminium treated rabbits showed a significant decrease in blood haemoglobin (Hb), Total Erythrocytic Count (TEC) and Packed Cell Volume (PCV) and increased Total Leukocyte Count (TLC). Luty et al., (2001) reported that irrespective of the dose, the deltamethrin and fenvalerate stimulated erythropoiesis and synthesis of Hb in Swiss mice. The non-significant effect of pyrethroid insecticide was observed by the Saka et al., (2011) in RBC, PCV and Hb. This study reveals that the RBC, PCV and Hb were higher in the test groups when compared with control rats. The PCV values are important in determining the effect of stress on the health of animals and indicate of oxygen carrying capacity of the blood (Larson et al., 1985). MCV, MCH and MCHC decreased in all treated groups compared to control group in the present study. The blood indices like MCV, MCH and MCHC have a particular importance in anaemia diagnosis in most animals. They tell the particular type of anaemia based on RBC size and relative Hb content. Results from this study imply that lambda cyhalothrin do alter neither RBC size nor relative Hb content. Ratnasooriya et al., (2005) reported that high dose of ICON (Lambda cyhalothrin) significantly lowered RBC count, the PCV, the MCHC and pg respectively. Lambda cyhalothrin treated mice obviously became progressively anaemic. The experiment was evidence by significant decrease in RBC count, Hb and PCV levels in comparison to control mice. Decrease in MCV, MCH and MCHC was observed in mice treated with Lambda cyhalothrin (Mosbah, 2010; Fetoui, 2008) in rats treated with cypermethrin (Institoris et al., 1999; Sayim et al., 2005).
The increase in WBC was noted in Lambda cyhalothrin treated mice compared to the control group. An increase in the number of leukocytes in the blood of animals – irrespective of the pyrethroid applied for intoxication – may result from the mobilization of the immunological system and/or a shift in the leukocytic pool from the spleen to peripheral blood (Luty et al., 2000; Maj, 2002). The increase in WBC may be indicative of activation of defense and immune system of the body (Yousef et al., 2003). This might result an increase in release of WBC from bone marrow storage pool into the blood. Pathological leukocytosis may have resulted due to chemical, acute haemorrhages and acute haemolysis. Leukocytosis may have occurred due to resistance of the animal for localization of inflammatory response (Benjiman, 1978). The rise in WBC count suggests the increased defence mechanism against probable attack of toxic molecules.

Increased WBC count was observed in mice treated with Lambda cyhalothrin (Mosbah, 2010; Fetoui, 2008), BHC (Philips, 1984), alpha-cypermethrin (Luty et al., 2000), deltamethrin and fenvalerate (Luty et al., 2001), pyrethroids like alpha-cypermethrin, deltamethrin and fenvalerate (Maj, 2002). Basir et al., 2011 noted that a blood analysis of rabbits treated with lambda-cyhalothrin revealed a significant decrease in red blood cell and white blood cell counts, haemoglobin concentration and lymphocytes, while mean corpuscular haemoglobin concentration, mean corpuscular volume, neutrophils, monocytes and eosinophils all increased. The present study suggests that pyrethroid Lambda cyhalothrin must be examined for their possible adverse effects on animals and humans before their application to agricultural fields.

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