Evaluation of Acute toxicity of Lambda Cyhalothrin in Mus musculus L.

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Lambda Cyhalothrin (LCT) is a type II synthetic pyrethroid widely used in agriculture, home pest control and protection of food stuff. Here, we evaluated its toxicity on biochemical parameters (Total protein, Acetyl cholinesterase, RNA and DNA) and liver histological alteration in mice after 24 h of oral administration @ 25, 50 and 75% of LD_{50} i.e., 26.49 mg/kg/body wt. Distilled water (DW) and Cyclophosphamide (CP @ 40 mg/kg/body wt.) were used as negative and positive control, respectively. LCT treated mice showed significant decrease in total protein (P <0.01), acetyl cholinesterase (P <0.001) and DNA (P <0.001) in a dose dependent manner. On the contrary, RNA content showed significant increase (P <0.01) at 50% of LD_{50} of LCT. Histological observations of the mice liver showed vascular congestion and hepatocyte degeneration with 6.63 mg/kg/body wt. of LCT; and accumulation of RBCs with sinusoid degeneration and wide necrotic area with pyknosis with 13.25 and 19.88 mg/kg/body wt. respectively. The results demonstrated LCT induced biochemical changes and hepatotoxicity in female mice.

Keywords: Cyclophosphamide (CP), House mouse, Insecticides, LCT, Pesticides, Pyrethroid toxicity

Synthetic pyrethroids have emerged as a new class of agricultural pesticides dominating organochlorine and organophosphate pesticides due to their high toxicity to a wide range of insects1. Lambda Cyhalothrin (LCT), a cyano-3-phenoxybenzyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate, is a potent synthetic type II pyrethroid having systemic and contact toxic effects against cockroaches, mosquitoes, ticks and flies, etc.2-4. Pyrethroids disrupt normal functioning of the nervous system in organisms resulting in paralysis or death5. LCT is relatively photo stable under natural irradiation and strongly adsorbed by particulates and plants. Adsorbed LCT molecules show decreased degradation rates because they are less accessible to breakdown than free molecules in the water column. The aerobic soil and aquatic degradation half-life of LCT is 42 and 21.9 days, respectively revealing persistence in the environment and increased chances of exposure to non target organisms including farmers6.

LCT exhibit toxic effects on non target organisms, particularly, vertebrates including mammals7-13. Reports on LCT induced biochemical and genetic toxicity are not uncommon9,11-13. LCT has been reported to induce histopathological alterations in mice10,11.

In the present study, we analyzed some biochemical (total protein, acetylcholinesterase activity, DNA and RNA) and histological parameters in female mice Mus musculus L. on acute toxicity induced by different sublethal doses of LCT.

Materials and Methods

Procurement and maintenance of animals—Healthy experimental Swiss albino female mice (Mus musculus L.), aged 7–8 wk were procured from Lala Lajpat Rai University of Veterinary Science, Hisar, Haryana, India and were acclimatized to the laboratory environment at 22±3°C, 50-60 % RH, and a 12 h L:D cycle for 2 wk. All animals were housed in polypropylene cages and given food and water ad libitum.

Dose schedule—The study design on mice was approved by the Institutional Animal Ethics Committee, Maharshi Dayanand University, Rohtak, Haryana. Mice were randomly divided into five treatment groups each consisting of five mice. The first group served as a control and was administered with distilled water (DW); group II with Cyclophosphamide (Himedia) @ 40 mg/kg body wt. of mice. The other three groups were given Lambda Cyhalothrin (5% EC) @ 6.63, 13.25 and 19.88 mg/kg body wt., respectively. CP, as recommended by OECD, served as positive control due to its cytotoxic alkylating nature. It produced highly active carbonium ion which reacts with the extremely electron rich area of nucleic acids and proteins14. The toxicity induced by CP was compared with DW and different doses of LCT treated mice. The tested doses were administered by oral gavage. At the end of 24 h exposure the mice were sacrificed by cervical dislocation after light anesthesia.
Biochemical studies—Blood samples were collected by cardiac puncture in EDTA tubes for estimation of total protein and acetylcholinesterase. These tests were done using Avecon Analyzer with Avecon Commercial Kit. The samples for estimation of DNA and RNA were taken from liver tissue as liver is the first organ exposed to pesticides and act as a huge complement of detoxification machinery system. All the samples were taken in triplicate. Tissue samples were thoroughly washed in normal cold saline (4–6°C), blotted dry, weighed, and homogenized (10% w/v) in 50 mM Tris-HCl buffer (pH 7.5) with a teflon-coated pestle. The homogenates were centrifuged at 4°C for 10 min at 3000 rpm in a refrigerated centrifuge and supernatants were either used fresh or kept frozen at −20°C until further use. Total DNA was estimated by Diphenylamine method using DNA (Himedia) as a standard, whereas total RNA was estimated by the Orcinol method using RNA (Himedia) as a standard as described by Schneider.

Histological examination—For histopathological examination, liver was removed and transferred to fresh 10% neutral buffered formalin for 24 h fixation and then processed for paraffin embedding. After routine processing, paraffin sections of liver tissue 5-6 micron were microtomed and stained with Haematoxyline and Eosin (H & E) for histological observation.

Statistical analysis—The statistical calculations (mean, standard deviation, standard error, t-test, etc.) were followed using Statistical Stat Soft Inc., Release 5.0, Tulsa, OK, USA.

Results and Discussion

Lambda Cyhalothrin was registered by the U.S. Environmental Protection Agency in 1988 and classified as class II toxic material. LCT affects the entire nervous system and preferred for large scale uses due to their higher potency. It penetrates the insect cuticle disrupting nerve conduction within minutes leading to cessation of feeding, prolonged nervous system depolarization and hyperexcitation. LCT is axonic poison and binds to a protein that regulates the voltage-gated sodium channel and prevents it from closing normally, which results in continuous nerve stimulation leading to paralysis.

In present study, the LCT administered mice showed hyper-excitability, abnormal facial sensation, irritability and seizures, decreased intake of food and tremors. Behavioural symptoms observed were severe @ 19.88 mg/kg body wt.

Biochemical studies—Changes in the biochemical parameters are presented in Table 1. A significant decrease in TP (36.36%, *P < 0.01), AChE (61.9%, *P < 0.001) and DNA (58.45%, *P < 0.001) at 75% of LD₅₀ of LCT was observed. The reduction in protein, DNA and acetylcholinesterase level in the pesticide treated group was in dose dependant manner. The RNA increased significantly at medium (44.94%, *P < 0.01) as well as high dose (37.80%, *P < 0.05) but insignificant @ 6.63 mg/kg body wt.

Histological observations—In the control group, a normal cellular morphology, distinct hepatocytes, sinusoidal spaces, and central vein were observed (Fig. 1A). The CP treated (40 mg/kg body wt.) group showed enormous extent of leukocyte inflammatory cell, elongated central and portal vein and hepatocyte degeneration (Fig. 1B). However, microscopic examination of LCT treated mice liver revealed many dose dependent degenerative changes. In the low-dose (group 3) 25% of LD₅₀ of LCT, vascular congestion and hepatocyte degeneration were observed (Fig. 1C). Further, accumulation of RBCs with sinusoid degeneration and wide necrotic area with pyknosis was observed in the medium dose (50% of LD₅₀) and high dose (75% of LD₅₀) treated tissue (Fig. 1D and E), respectively.

In addition, we observed significant decrease in total protein content (Table 1) which might be due to...

Table 1—Effect of Lambda Cyhalothrin on different biochemical parameters of female mice.

<table>
<thead>
<tr>
<th></th>
<th>CP (40 mg/kg body wt.)</th>
<th>Control (DW)</th>
<th>LCT (mg/kg body wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Protein</td>
<td>4.39±0.17</td>
<td>7.81±0.39</td>
<td>6.63</td>
</tr>
<tr>
<td>AChE (U/L)</td>
<td>2.36±0.07</td>
<td>6.64±0.24</td>
<td>6.20±0.09*</td>
</tr>
<tr>
<td>DNA (µg/ml)</td>
<td>0.67±0.04</td>
<td>1.46±0.10</td>
<td>4.45±0.17**</td>
</tr>
<tr>
<td>RNA (µg/ml)</td>
<td>1.74±0.09</td>
<td>1.12±0.15</td>
<td>1.08±0.03*</td>
</tr>
</tbody>
</table>

Level of significance: *P < 0.05, ** P < 0.01 and *** P < 0.001
the impairment of protein synthesis and necrosis of hepatocyte cells. Earlier workers have shown that the quantity of protein could be affected by destruction or necrosis of cells and impaired incorporation of amino acids into polypeptide chains. Decrease in total protein might be due to damages in liver caused by LCT as reported earlier that hepatotoxicity results in decreased serum protein in rat. Reduction in total protein has been reported already in other animal models viz., Zebrafish and Catfish.

Similarly, we have observed significant reduction of AChE activity in the LCT induced groups in a dose dependant manner. The impairment of AChE activity has been reported to be one of the LCT neurotoxicity mechanisms in vertebrates as well as insects; and occupation of its active sites by pollutants may lead to decreased cellular metabolism and thereby disturbed metabolic and nervous activity. A dose dependent reduction of AChE activity has been already shown in rats exposed to LCT @ 4.2 and 8.4 mg/kg body wt. for 4 wk in brain and other tissues, respectively. The inhibition induced by pyrethroids could be attributed to their lipophilicity, whereby they could penetrate the cell membrane easily.

Further, in the present study, we observed alterations in both DNA and RNA content in mice liver exposed to tested doses of LCT and CP. The DNA degradation is may be due to reactive oxygen species as has been reported to be generated during LCT metabolism and produce oxidative stress. Other reason may be the electrophilic epoxide produced which are responsible for toxicity induction. The later has a tendency to react with electron rich molecule in the DNA and gives rise to DNA degradation. Such decreased DNA content has been earlier reported in Zebrafish. Contrarily, the DNA content of freshwater fish liver has been reported to be significantly elevated in response to the LCT. On the other hand, RNA content was observed to be increased in LCT treated and positive control groups. Sharma et al. reported that LCT exposed blood lymphocyte showed increased DNA and RNA content at different doses. However, Kumar et al. and Ahmad et al. have reported LCT induced RNA contents decline in Spotted snakehead and Zebrafish, respectively. The increased RNA content observed here could be the result of the efforts of the treated animals’ proliferations of some stress specific gene activation to cope up with active stress caused by LCT exposure.

Liver is considered as target organ of metabolism, detoxification and biotransformation by which a toxic compound is transformed into less harmful form with reduced toxicity. Pyrethroid insecticides are lipophilic
compounds that are typically absorbed through the gastrointestinal and respiratory tracts and also tend to partition into lipid-rich internal tissues, including liver. In present study, liver showed vascular congestion, hepatocyte degeneration, vacuolisation, sinusoid degeneration, enlargement of portal and central vein. These results are in accordance with Al-Sarar et al., in which histological changes in male mice exposed to LCT at various sublethal dose of 0.2, 0.4, 0.8 mg/kg/day in chronic (6 wk) study revealed vascular congestion and degeneration, focal mononuclear cell, areas of degeneration and wide necrotic areas. The extent of damage was more (enlargement of central and portal vein, sinusoid degeneration) in our study which could be due to the difference in doses of LCT and nature of treatments. Further, in consonance congestion of hepatic blood vessels and sinusoids and vascular degeneration of hepatic cells with nuclear changes in liver of male mice exposed to LCT @ 9.5 mg/kg and 2.37 mg/kg body wt. has been reported. Similar effects have also been reported in rats and rabbits.

The overall results of this study further established the toxicity of Lambda Cyhalothrin (LCT) and suggests restricted application of this pesticide, if not complete avoidance.

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