Beta-Cyfluthrin Induced Histochemical Alterations in the Liver of the Albino Rat

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Summary
Pesticide poisoning and related diseases have gained considerable attention in the recent past. Beta-cyfluthrin is a type II pyrethroid pesticide and is widely used against crop and house hold pests. The present study has been designed to assess histochemical alterations following beta-cyfluthrin intoxication in the liver of the female albino rat (Rattus norvegicus). Beta-cyfluthrin was orally administrated, 35.48 mg/kg b.wt. for acute (1 day) and 5.06, 2.53 and 1.68 mg/kg b.wt. for sub-acute (7, 14 and 21 days) treatments respectively. Beta-cyfluthrin caused marked hepatotoxicity in the form of altered hepatosomatic index viz. liver weight, body weight and liver weight-body weight ratio and histochemical localization of DNA and proteins in the liver of albino rat. On the basis of histochemical study, it could be revealed that beta-cyfluthrin causes a reduction in hepatic proteins and an enhancement in the hepatic DNA. The centrilobular zone was the zone of maximum alteration followed by the midzone and periportal zone of the hepatic lobule. Decreased protein content is a consequence of lysis of structural proteins and the need to support increased cell proliferation under stress of beta-cyfluthrin, whereas increased DNA is a consequence of increased mitogenic activity resulting in excessive cell proliferation. It is proposed that xenobiotic metabolising enzymes, which are most concentrated in the centrilobular zone, cause the formation of metabolites of beta-cyfluthrin, which are toxic to the liver. Lower oxygen levels in the centrilobular zone, compared to other parts of the liver lobule, may have also contributed to the observed histochemical changes being greatest in the centrilobular zone.

Introduction
Pesticides are presently a cynosure both among farmers as well as the toxicological community. The mighty role of pesticides to farmers surely merits appreciation but their widespread use is associated with serious health hazards to various non-target organisms (Bian et al., 2004; Bhalli et al., 2006; Farrag & Shalby, 2007).

Regardless of the ill effects, unsafe and indiscriminate pesticidal use is widespread in developing and underdeveloped nations. Owing to poor economic conditions in these countries, agriculture is still the mainstay of the economy, which in turn plays a key role in continuous pesticidal use (Patil et al., 2003; Jamil et al., 2007; Narendra et al., 2008). Presently, pyrethroids account for about 25% of the total pesticidal market (Anand et al., 2006; McCarthy et al. 2006). Attributes favouring use of pyrethroids include limited soil persistence, photostability and high insect/mammalian ratio (Atessahin et al.; 2005; Singh et al., 2005). Pyrethroids are broadly divided into type I and type II, based on absence and presence of cyano group respectively and behavioural profiles (Sayim et al., 2005). Beta-cyfluthrin is a type II pyrethroid pesticide with a fluorine atom attached to position 4 in its structure and having broad potential against target as well as non-target species (Cox, 1994; Khambay, 2002; Omotuyi et al., 2006).

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The present study has been undertaken to assess histochemically qualitative alterations in hepatocytic DNA and proteins under stress of beta-cyfluthrin.

Materials and Methods
Experimentation was conducted on 40 adult female albino rats, *Rattus norvegicus* (Berkenhout). These animals were kept in standard polypropylene cages at a temperature of 25±5°C and a photoperiod of 10 hours/day. Rats were fed on Goldmohar brand rat feed procured from Hindustan Uni Lever Ltd., Mumbai and provided water *ad libitum*. After 10 days of acclimatization these animals were divided into 8 groups, 4 serving as treated and other 4 as control corresponding to each treated set. Experimental compound, beta-cyfluthrin was obtained from Bayer India Ltd., Mumbai and its calculated LD₅₀ is 354.8 mg/kg/b.wt (*Singh, 2003*). Albino rats weighing 100±5 gm were administered an acute dose (35.48 mg/kg b.wt.) and sub-acute dose (5.06, 2.53 and 1.68 mg/kg b.wt.) of beta-cyfluthrin by gavage tube for acute (1 day) and sub-acute (7, 14 & 21 days) treatment respectively. Animals were autopsied at predetermined time intervals, quickly dissected and liver tissue was excised out. Liver was soon weighed, sliced and fixed in Carnoy’s fixative (*Gatenby and Beams, 1950*). Fixation was followed by dehydration, embedding in wax and sectioning (*Pearse, 1980; Humason, 1979*). Sections of 5 micrometer thickness were taken on clean slides, stained with mercuric bromophenol blue (*Chapman, 1975*) for localization of hepatocytic proteins and Feulgen reaction (*Feulgen & Rossenback, 1924*) was conducted for histochmical localization of DNA in liver. Quantitative aspects viz. body weight, liver weight and liver weight-body weight ratio (hepatosomatic index) were also considered and student’s ‘t’ test (*Fisher & Yates, 1950*) was conducted to assess statistical significance.

Results
The investigations have demonstrated histochmically a decrease in hepatic protein following acute (1 day) as well as sub-acute (7, 14 & 21 days) beta-cyfluthrin intoxication, on the contrary a simultaneous increase of hepatic DNA has been observed. The lobule is the functional unit of liver and contains three zones viz. centrilobular, midzonal and periportal zone. Our observations revealed the centrilobular zone to be the most sensitive, followed by midzone & periportal zone, with regard to the effect of beta-cyfluthrin on proteins and DNA (Plate-Ia-Ie; Plate-IIa-IIe).

Plate-I(a): Histochemical localization of hepatic DNA in control albino rat (400X)
Plate-I(b): Histochemical localization of hepatic DNA in albino rat after acute (1day) beta-cyfluthrin intoxication (400X)
Plate-I(c): Histochemical localization of hepatic DNA in albino rat after sub-acute (7days) beta-cyfluthrin intoxication (400X)
Plate-I(d): Histochemical localization of hepatic DNA in albino rat after sub-acute (14days) beta-cyfluthrin intoxication (400X)
Plate-I(e): Histochemical localization of hepatic DNA in albino rat after sub-acute (21days) beta-cyfluthrin intoxication (400X)
Quantitative aspects viz. body weight, liver weight & liver weight-body weight ratio (hepatosomatic index) were found to increase significantly in treatment groups (Table-1).

**Table 1.** Body weight, liver weight and liver weight-body weight ratio of *Rattus norvegicus* after acute (1 day) and sub-acute (7, 14 and 21 days) beta-cyfluthrin intoxication

<table>
<thead>
<tr>
<th>Treatment time (in days)</th>
<th>Treatment</th>
<th>Dose (mg/kg b.wt.)</th>
<th>No. of rats</th>
<th>Body weight (gm) Mean±S.E.</th>
<th>Liver weight (gm) Mean±S.E.</th>
<th>Liver wt.-Body wt. ratio Mean±S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day</td>
<td>Control</td>
<td>-</td>
<td>5</td>
<td>109.7±2.90</td>
<td>3.17±0.11</td>
<td>0.028±0.0002</td>
</tr>
<tr>
<td></td>
<td>Acute</td>
<td>35.48</td>
<td>5</td>
<td>113.3±3.28*</td>
<td>3.25±0.09*</td>
<td>0.029±0.0003*</td>
</tr>
<tr>
<td>7 days</td>
<td>Control</td>
<td>-</td>
<td>5</td>
<td>112.0±1.73</td>
<td>3.28±0.12</td>
<td>0.029±0.0006</td>
</tr>
<tr>
<td></td>
<td>Sub-acute</td>
<td>5.06</td>
<td>5</td>
<td>120.7±2.40**</td>
<td>3.40±0.01*</td>
<td>0.028±0.0004*</td>
</tr>
<tr>
<td>14 days</td>
<td>Control</td>
<td>-</td>
<td>5</td>
<td>116.3±1.20</td>
<td>3.38±0.03</td>
<td>0.029±0.0004</td>
</tr>
<tr>
<td></td>
<td>Sub-acute</td>
<td>2.53</td>
<td>5</td>
<td>124.0±2.31**</td>
<td>3.76±0.09***</td>
<td>0.030±0.0007*</td>
</tr>
<tr>
<td>21 days</td>
<td>Control</td>
<td>-</td>
<td>5</td>
<td>120.0±2.31</td>
<td>3.46±0.01</td>
<td>0.030±0.0004</td>
</tr>
<tr>
<td></td>
<td>Sub-acute</td>
<td>1.68</td>
<td>5</td>
<td>127.3±0.03***</td>
<td>3.79±0.09****</td>
<td>0.031±0.005**</td>
</tr>
</tbody>
</table>

* P>0.05, ** P<0.05, *** P<0.01, ****P<0.001

**Discussion**
DNA has a central role due to its unique functions and is present mainly on chromosomes in association with nucleoproteins in the nucleus of mamma-
lian cells. In addition to being part of chromosomes, proteins play a significant role in structure and metabolism of the cell. The bulk of the proteins within the cell is present in cellular as well as subcellular membranes. Membrane proteins primarily aid to maintain integrity of the cell & cellular organelles, with a secondary function being selective passage of materials through channels & pumps (Lodish et al., 2001). The liver is a hub for protein synthesis and also a target organ for xenobiotics due to its detoxifying function and unique relationship to the gastrointestinal tract. Beta-cyfluthrin probably produces its toxicity in a two step manner, initially due to affinity towards certain components of cell membranes causing membrane damage and later by forming more toxic intermediates (Vandenberghhe, 1996).

Decrease in proteins as observed histochemically in this investigation[Plate II(a)-II(e)] may be a result of damage through both these steps. Initial damage to proteins includes lysis of structural proteins whereas secondary damage may be at genetic level by altering cell cycle leading to proliferation of hepatic cells. (Sakr et al. 2004; Manna et al., 2004; Ksheerasagar & Kaliwal, 2006; Omotuyi et al. 2006).

Qualitative enhancement of hepatic DNA has been found histochemically following acute as well as sub acute beta-cyfluthrin intoxication. DNA is the genetic material of the cell and although enhanced DNA is suggestive of increased mitogenic activity, it is noteworthy that beta-cyfluthrin has been reported to have genotoxic effects (Rana et al., 2008). Qualitative decrease in hepatic proteins is an indicator of damage to membrane integrity, which in turn gives a clue to the entry of toxicant into the cell. Within the cell, both toxicant as well as its metabolic intermediates can cause a variety of damage including the release of various hydrolytic enzymes (e.g. nucleases and proteases). Cells with damage to these enzymes are very prone to chromosomal damage and altered cell cycle (Singh & Saxena, 2002). Beta-cyfluthrin might have caused excessive DNA synthesis in the present study due to altered mitogenic activity (Kolaja et al. 1996; Kostka et al. 1996). Significant increase in liver weight and liver weight-body weight ratio is also an indicator of hepatocellular proliferation under stress of beta-cyfluthrin and may have increased due to excessive cell division (Shakoori et al. 1988; Gupta & Kumar, 1991; Singh et al., 2005). Body weight has been found to increase which may be due to altered CNS functions as evident in the form of excessive food intake in treated sets (Ahmed et al., 1989; Sharma, 1997; Kumar, 2001; Singh, 2003).

Within the three zones of a hepatic lobule, the presence of both DNA & proteins has been found to be altered greatly in the centrilobular zone but less so in the midzone and perportal zone. This may be due to the fact that when blood flows from portal triad towards the central vein, oxygen content goes on decreasing towards the successive zones and also most of the xenobiotic metabolising enzymes are concentrated in the centrilobular and midzone. There must be thus the formation of toxic intermediates during processing in these zones, which is responsible for pronouncing the effect in these regions (Anadon et al., 1995; Hinton, 2000).

The above facts strongly suggest that beta-cyfluthrin has the potential to disrupt normal hepatic functions in mammals and may lead to more deleterious consequences. These facts thus advocate the necessity of proper precautions to be taken during use of this pesticide.

References


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