Chapter II

DETERMINATION OF LD$_{50}$ OF ALKALI SOLUBILIZED
Bacillus thuringiensis subsp. israelensis δ-ENDOTOXIN
Introduction

LD₅₀ value of δ-endotoxin was estimated by the ‘Acute Toxicity Test’. The LD₅₀ is a statistical estimate of the acute lethality of an agent administered to a specified sex, age and strain of a species of animal. The purpose of the LD₅₀ study is an important investigation to allow the investigator to assess the relative toxicity of an unknown compound which might follow the administration of a single dose (or an overdose).

Acute toxicity study of a new compound must be performed accurately during its pharmacological and/or toxicological screening. This study is carried out on animals in the laboratory applying sophisticated procedure. A quantitative aspect of acute toxicity testing is the determination of lethal dose of a compound. This is usually expressed as LD₅₀. During the acute phase of the study, the information gleaned from the experiment includes not only a rapid assessment of lethality, but other signs and symptoms of acute toxicity, possible target organs and some minimal informations which in turn, will assist investigators in designing subsequent subchonic and chronic studies of a compound.

It is usual to calculate the lethal dose after administration of the compound by several routes, but most attention will naturally be directed towards the effects caused by the mode of administration which most nearly approaches that by which the compound will be given.

Determination of the LD₅₀ demands the use of the relatively large number of animals and it is customary to make an accurate determination on one species (the rat or mouse) only. LD₅₀ value of δ-endotoxin was determined intraperitoneally in mice. The advantage of the LD₅₀ study at an early stage is a comparative one, allowing the investigator to assess the relative toxicity of an unknown agent in terms
of toxicity associated with other agents tested in the same species (strain, sex and age) as well as the doses used to establish the agent’s spectrum of toxicological activity can be related to its lethal dose.

**Materials and Methods**

Solubilization and extraction of δ-endotoxin from dry cell biomass (JUS-3) as well as the estimation of the toxin potency were performed in our laboratory by the method described in chapter I. The quantitation of δ-endotoxin is the results of average of six experiments and the potency is expressed in kilo international toxic unit (kitu) [210]. The δ-endotoxin was diluted with normal saline. Inbred swiss mice of either sex weighing between 20 to 25 g were used. Animals were allowed to free access for standard pellet food (Hindusthan lever) and drinking water *ad libitum*. Animals were not provided food for last 18 hrs. before the commencement of experiment. The experiment was performed with two groups at each dose level, one for vehicle control (physiological saline) and other for the δ-endotoxin.

The LD$_{50}$ value of δ-endotoxin was derived by the method of Litchfield and Wilcoxon [211]. In the interpretation of the toxicity (LD$_{50}$), the observed percentage mortality was converted into probit by referring to the appropriate table [212]. The values thus obtained were plotted against the corresponding log dose. Results were made fitted with straight line after regression analysis of probit. The dose corresponding to probit 5 was found to be the LD$_{50}$ [212].

**Results and Discussion**

Vehicle control mice showed no mortality at any dose level, so the data associated with vehicle treatment were not inserted in the table, results of the δ-endotoxin has been summarised in the table 2.1. From figure 2.1. LD$_{50}$ value of the δ-endotoxin has been found to be log 2.74 and its antilog is 549.54. Therefore, intraperitoneally the LD$_{50}$ value of δ-endotoxin of *Bacillus thuringiensis* subsp. *israelensis* in mice is 549.54 kitu/kg body weight.
### TABLE 2.1. Results of acute intraperitoneal toxicity of δ-endotoxin

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (Kitu/kg b.t.w.)</th>
<th>Log dose (x)</th>
<th>No. of animals</th>
<th>No. of dead</th>
<th>No. of survival</th>
<th>Dead (%)</th>
<th>Probit (y)</th>
<th>$x^2$</th>
<th>$xy$</th>
<th>Expected probit (Y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>400</td>
<td>2.602</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>3.72</td>
<td>6.770</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>420</td>
<td>2.620</td>
<td>10</td>
<td>1</td>
<td>9</td>
<td>10</td>
<td>4.16</td>
<td>7.086</td>
<td>6.846</td>
<td>9.740</td>
</tr>
<tr>
<td>3.</td>
<td>460</td>
<td>2.662</td>
<td>10</td>
<td>2</td>
<td>6</td>
<td>40</td>
<td>4.75</td>
<td>7.279</td>
<td>12.815</td>
<td>4.80</td>
</tr>
<tr>
<td>4.</td>
<td>500</td>
<td>2.698</td>
<td>10</td>
<td>4</td>
<td>5</td>
<td>50</td>
<td>5.00</td>
<td>7.463</td>
<td>13.660</td>
<td>4.90</td>
</tr>
<tr>
<td>5.</td>
<td>540</td>
<td>2.732</td>
<td>10</td>
<td>5</td>
<td>4</td>
<td>60</td>
<td>5.25</td>
<td>7.634</td>
<td>14.505</td>
<td>5.20</td>
</tr>
<tr>
<td>6.</td>
<td>580</td>
<td>2.763</td>
<td>10</td>
<td>6</td>
<td>3</td>
<td>70</td>
<td>5.52</td>
<td>7.795</td>
<td>15.412</td>
<td>5.50</td>
</tr>
<tr>
<td>7.</td>
<td>620</td>
<td>2.792</td>
<td>10</td>
<td>7</td>
<td>0</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>640</td>
<td>2.806</td>
<td>10</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td>7.873</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ \xi x = 21.675 \]
\[ \bar{x} = 2.709 \]
\[ \xi y = 28.4 \]
\[ \bar{y} = 4.73 \]
\[ \xi (x)^2 = 469.805 \]
\[ \bar{x} = 58.72 \]
\[ \xi x \times \xi y = 21.675 \times 28.4 \]
\[ \bar{x} = 615.57 \]
\[ \xi x^2 = 76.946 \]

\[ b = \frac{\xi xy - \xi x \times \xi y}{\xi x^2 - \frac{(\xi x)^2}{n}} = \frac{77.212 - 76.946}{65.334 - 58.72} = 0.04 \]

b is known as regression coefficient

Linear regression equation:

\[ Y = \bar{y} + b(x - \bar{x}) \] where \( \bar{x} \) and \( \bar{y} \) are the mean values of x and y, i.e. \( Y = 4.73 + 0.04(x - 2.709) \)
Fig. 2.1. Graphical method of determination of LD$_{50}$ of δ-endotoxin in mice (I.P.)