6.1 MATERIAL AND METHODS

6.1.1 Source of live animals
Four month old 100 healthy Wistar rats (male and female both) obtained from the Guru Angad Dev veterinary and animal sciences, Ludhiana (Pb.) were used. Rats were placed in propylene cages and given water ad libitum and rat-pelleted diet (Amrut Pvt. Ltd). The Institutional Animal Ethics Committee (IAEC) of Baba Isher Singh College of Pharmacy, Gagra, Moga (Reg. No. 766/2007/2455/26/38/CPCSEA), approved the experimental protocol (Ref. no. BIS/COP/IAEC/01) and the care of laboratory animals was taken as per the guidelines of CPCSEA, Ministry of Forests and Environment, Government of India.

6.1.2 Chemicals
Cadmium chloride, Sodium Chloride

6.1.3 Acute toxicity studies
24 wistar rats were weighed 140-150 g each were used for this study. The animals were fasted for 12 h before the study, but were allowed water ad libitum. In the first trial, four groups (n=3) were given normal saline as control group and 10, 100 and 10,00 mg/kg of the extract orally for the remaining three groups respectively. They were then observed for 24 h for signs of toxicity or deaths. In second trial, another four groups (n=3) were given normal saline, 2000, 4000 and 8000 mg/kg of extract orally for the remaining groups respectively and were observed for 24 h for signs of toxicity or deaths and acute toxicity study was carried out according to OECD guidelines. The median lethal dose (LD$_{50}$) was calculated.$^{162}$

6.1.4 Animals and experimental setup
Fifty Wistar rats of both sexes weighing 145-160 gm. were used for the study the effects of aqueous extract of *Tecomella undulata* on spleen of the animals. They were kept in standard propylene cages at 25$^\circ$C and 12 h light/dark condition in the animal room of the Department of Pharmacology, BISCOP, Gagra. They were fed on commercial rat’s feeds
and were given water ad libitum. The animals were fasted from feeds for 12 h before the commencement of each experiment, but were allowed water ad libitum. The experimental setup was done for two times, firstly for testing the activity of extract and secondly for testing of prepared phytosomal syrup on wistar rat. Oral route was selected for testing of both extract and phytosomal syrup.

For testing of bark extract and Phytosomal syrup of *Tecomella undulata* the rats were divided into three large groups and received the following treatment:

- **Group I** – Standard food only 50 g
- **Group II** – Cadmium chloride 10 ml of 20 ppm solution mixed with 50 g of food
- **Group III** – Tecomella undulata extract / Phytosomal syrup of *Tecomella undulata* + cadmium chloride 10 ml of 20 ppm solution mixed with 50 g of food.

In each group 10 rats were used and the experiment was done three times. Blood sample was taken from rats of all the batches by puncturing of heart tissue on 31st day for complete blood count and then rats of the entire groups were killed.

Prior to fixation in Bouin’s fluid, the spleen was measured in each group for its length and width. For studying the internal structure of spleen tissues photomicrographs showing spleen cells were taken. For photo-micrographic study, fixed spleen was cut at 6 μ and stained in Delafield’s hematoxylin and eosin.¹⁶³⁻¹⁶⁴

**6.1.5 Statistical analysis**

Size of spleen was expressed in centimeters (mean ± SEM). The data were statistically analyzed using ANOVA with multiple comparisons versus control group by Dennett’s method. Values of *p*<0.05 or less were taken as significant.
6.2 RESULTS

6.2.1 Acute toxicity study
The mortality rates for 10, 100, 1000, 2000, 4000 and 8000 mg/kg of the extract was 0, 0, 0, 0, 51 and 100%. The LD$_{50}$ was calculated as 3920 mg/kg body weight of wistar rat.

6.2.2 In-vivo study of plant extract

6.2.2.1 Morphological study
Following CdCl$_2$ administration, spleens showed enlargement in size. It showed significant increase in the length and width of the spleen (55% and 61% respectively) as compared to the controls. When CdCl$_2$ was administered along with Tecomella undulata extract and food, the spleen showed minor difference in length and width, thus indicating almost normal size and shape. (Table 6.1, 6.2)

Table 6.1: Effect of cadmium chloride alone and in combination with extract of T. undulata on the morphology of rat spleen’s length

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Treatment</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (A)</td>
<td>16.651±0.9298</td>
</tr>
<tr>
<td>2</td>
<td>Cdcl$_2$ group (B)</td>
<td>26.861±1.374</td>
</tr>
<tr>
<td>3</td>
<td>Cdcl$_2$ + Aq. Extract of TU (C)</td>
<td>17.185±0.4928</td>
</tr>
</tbody>
</table>

Significant difference at 5% level of significance using Student’s ‘t’ test.

Table 6.2: Anova table describe the distribution of a group of values of length of rat’s spleen for column comparison and descriptive statistics for each column

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mean</td>
<td>16.651</td>
<td>26.861</td>
<td>17.185</td>
</tr>
<tr>
<td>2</td>
<td>Standard deviation (SD)</td>
<td>0.9298</td>
<td>1.374</td>
<td>0.4928</td>
</tr>
<tr>
<td></td>
<td>Sample size (N)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>----------------</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Std. error of mean(SEM)</td>
<td>0.294</td>
<td>0.4346</td>
<td>0.1558</td>
</tr>
<tr>
<td>5</td>
<td>Lower 95% conf. limit</td>
<td>15.986</td>
<td>25.878</td>
<td>16.833</td>
</tr>
<tr>
<td>6</td>
<td>Upper 95% conf. limit</td>
<td>17.316</td>
<td>27.844</td>
<td>17.537</td>
</tr>
<tr>
<td>7</td>
<td>Minimum</td>
<td>14.75</td>
<td>25.25</td>
<td>16.7</td>
</tr>
<tr>
<td>8</td>
<td>Median (50th percentile)</td>
<td>16.725</td>
<td>26.93</td>
<td>16.975</td>
</tr>
<tr>
<td>9</td>
<td>Maximum</td>
<td>18.15</td>
<td>28.8</td>
<td>18.2</td>
</tr>
<tr>
<td>10</td>
<td>Normality test KS</td>
<td>0.1426</td>
<td>0.2046</td>
<td>0.2463</td>
</tr>
<tr>
<td>11</td>
<td>Normality test P value</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>0.0867</td>
</tr>
<tr>
<td>12</td>
<td>Passed normality test?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Figure 6.1: Differences in length in spleen showing mean and standard deviation of three groups
Figure 6.2: Differences in length in spleen showing spot typographical errors on data

Table 6.3: Effect of cadmium chloride alone and in combination with cadmium chloride extract on the morphology of width of rat’s spleen

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Treatment</th>
<th>Width</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (A)</td>
<td>3.68±0.2124</td>
</tr>
<tr>
<td>2</td>
<td>Cdcl₂ group (B)</td>
<td>7.025±0.1568</td>
</tr>
<tr>
<td>3</td>
<td>Cdcl₂ + Aq. Extract of TU (C)</td>
<td>4.32±0.3335</td>
</tr>
</tbody>
</table>

Significant difference at 5% level of significance using Student’s ‘t’ test.

Table 6.4: Anova table describe the distribution of a group of values of width of rat’s spleen for column comparison and descriptive statistics for each column.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mean</td>
<td>3.68</td>
<td>7.025</td>
<td>4.32</td>
</tr>
<tr>
<td>2</td>
<td>Standard deviation (SD)</td>
<td>0.2124</td>
<td>0.1568</td>
<td>0.3335</td>
</tr>
<tr>
<td>3</td>
<td>Sample size (N)</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>--------------------------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>4</td>
<td>Std. error of mean(SEM)</td>
<td>0.06716</td>
<td>0.04958</td>
<td>0.1055</td>
</tr>
<tr>
<td>5</td>
<td>Lower 95% conf. limit</td>
<td>3.528</td>
<td>6.913</td>
<td>4.081</td>
</tr>
<tr>
<td>6</td>
<td>Upper 95% conf. limit</td>
<td>3.832</td>
<td>7.137</td>
<td>4.559</td>
</tr>
<tr>
<td>7</td>
<td>Minimum</td>
<td>3.35</td>
<td>6.8</td>
<td>3.85</td>
</tr>
<tr>
<td>8</td>
<td>Median (50th percentile)</td>
<td>3.7</td>
<td>7.05</td>
<td>4.275</td>
</tr>
<tr>
<td>9</td>
<td>Maximum</td>
<td>3.95</td>
<td>7.25</td>
<td>4.7</td>
</tr>
<tr>
<td>10</td>
<td>Normality test KS</td>
<td>0.1498</td>
<td>0.1873</td>
<td>0.2388</td>
</tr>
<tr>
<td>11</td>
<td>Normality test P value</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>12</td>
<td>Passed normality test?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**Figure 6.3:** Differences in width of spleen showing mean and standard deviation of three groups
Statistical analysis of the length and width of spleens among different groups revealed that CdCl₂ feeding caused splenomegaly, but when Tecomella undulata was administered along with CdCl₂, the spleen size remained normal.

6.2.2.2 Histological study

For histological study, fixed spleen was cut at 6 µ and stained in Delafield’s hematoxylin and eosin and prepared slides were observed under microscopy (Figure 6.5).
Figure 6.5 (a)
Normal spleen
10X

Figure 6.5 (b)
Normal spleen
40X
Figure 6.5 (c) Cadmium chloride group 10X: Photograph showing focal depopulation (white arrow), necrosis (blue arrow)

Figure 6.5 (d) Cadmium chloride group 40X: Photograph showing focal depopulation (white arrow), necrosis (blue arrow) & hyperplasia of white matter (black arrow)
Figure 6.5 (e)
Cadmium chloride + TU extract group
10X:
Photomicrograph showing normal sinus but some diffused pulp

Figure 6.5 (f)
Cadmium chloride + TU extract group
40X:
Photomicrograph showing normal sinus
Figure 6.5: Photomicrograph of rat spleen fixed with Bouin’s hematoxylin and eosin

In controls, rat spleen showed normal structure of the capsule; the trabeculi were normal, sinuses were well differentiated and the pulp was well organized (Figure 6.5 a). When CdCl₂ was fed to rat along with food, the spleen showed sinus congestion, with focal depopulation and hyperplasia of white matter. The pulp was organized but at places dead cells were seen. In the pulp most of the cells were swollen (Figure 6.5 d). Histology of the rat fed on CdCl₂ plus TU extract along with food showed normal capsule and trabeculi but the pulp was diffused. Sinuses were clear at many places (Figure 6.5 e).

6.2.2.3 Hæmatological study

The hematologic data of control group, cadmium chloride group, and drug treated wistar rats was given in table 6.5.
6.2.3 *In-vivo* study of phytosomal syrup

6.2.3.1 Morphology

Following CdCl$_2$ administration, their spleens showed enlargement in size. There was significant increase in the length and width of the spleen (56% and 89% respectively) as compared to the controls. When CdCl$_2$ plus phytosomal syrup of Tecomella undulata extract was administered to rat along with food, the spleen showed no difference in length and width, thus indicating normal size and shape. See the Table 6.6-6.9

Table 6.6: Effect of cadmium chloride alone and in combination with phytosomal syrup of aq. extract of tecomella undulata on the morphology of rat spleen’s length

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Treatment</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (A)</td>
<td>17.43+ 0.209</td>
</tr>
<tr>
<td>2</td>
<td>CdCl$_2$ group (B)</td>
<td>27.245+0.1334</td>
</tr>
<tr>
<td>3</td>
<td>CdCl$_2$ + Phytosomal syrup of aq. Extract of TU (C)</td>
<td>17.55+ 0.225</td>
</tr>
</tbody>
</table>

Significant difference at 5% level of significance using Student’s ‘t’ test.

Table 6.7: Anova table describe the distribution of a group of values of length of rat’s spleen for column comparison and descriptive statistics for each column.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Col. Title</th>
<th>Control Group</th>
<th>CdCl$_2$ Group</th>
<th>CdCl$_2$+Phytosomal syrup</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mean</td>
<td>17.43</td>
<td>27.245</td>
<td>17.55</td>
</tr>
<tr>
<td>2</td>
<td>SEM</td>
<td>0.209</td>
<td>0.1334</td>
<td>0.225</td>
</tr>
<tr>
<td>3</td>
<td>Sample size (N)</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>SD</td>
<td>0.6609</td>
<td>0.4219</td>
<td>0.4954</td>
</tr>
<tr>
<td></td>
<td>Lower 95% conf. limit</td>
<td>Upper 95% conf. limit</td>
<td>Minimum</td>
<td>Median (50th percentile)</td>
</tr>
<tr>
<td>---</td>
<td>----------------------</td>
<td>-----------------------</td>
<td>---------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>5</td>
<td>16.957</td>
<td>26.943</td>
<td>17.872</td>
<td>17.4</td>
</tr>
<tr>
<td>6</td>
<td>17.903</td>
<td>27.547</td>
<td>18.138</td>
<td>17.48</td>
</tr>
<tr>
<td>7</td>
<td>16.65</td>
<td>26.75</td>
<td>17.15</td>
<td>17.48</td>
</tr>
<tr>
<td>8</td>
<td>17.4</td>
<td>27.125</td>
<td>17.48</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 6.6:** Differences in length in spleen showing mean and standard deviation of three groups
Figure 6.7:  Differences in length in spleen showing spot typographical errors on data

Table 6.8: Effect of cadmium chloride alone and in combination with extract on the morphology of rat spleen’s width

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Treatment</th>
<th>Width</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (A)</td>
<td>3.77+ 0.2044</td>
</tr>
<tr>
<td>2</td>
<td>CdCl₂ group (B)</td>
<td>7.135+ 0.17</td>
</tr>
<tr>
<td>3</td>
<td>CdCl₂ + Phytosomal syrup of aq. Extract of TU (C)</td>
<td>3.905 + 0.4219</td>
</tr>
</tbody>
</table>

Significant difference at 5% level of significance using Student’s ‘t’ test.

Table 6.9: Anova table describe the distribution of a group of values of width of rat’s spleen for column comparison and descriptive statistics for each column.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Col. Title</th>
<th>Control Group</th>
<th>CdCl₂ Group</th>
<th>CdCl₂+Phytosomal syrup</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mean</td>
<td>3.77</td>
<td>7.135</td>
<td>3.905</td>
</tr>
<tr>
<td>2</td>
<td>Standard deviation (SD)</td>
<td>0.06464</td>
<td>0.05377</td>
<td>0.1334</td>
</tr>
<tr>
<td>3</td>
<td>Sample size (N)</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Column</td>
<td>Std. error of mean(SEM)</td>
<td>Lower 95% conf. limit</td>
<td>Upper 95% conf. limit</td>
<td>Minimum</td>
</tr>
<tr>
<td>--------</td>
<td>-------------------------</td>
<td>-----------------------</td>
<td>-----------------------</td>
<td>---------</td>
</tr>
<tr>
<td>A</td>
<td>0.2044</td>
<td>3.624</td>
<td>3.916</td>
<td>3.5</td>
</tr>
<tr>
<td>B</td>
<td>0.17</td>
<td>7.013</td>
<td>7.257</td>
<td>6.85</td>
</tr>
<tr>
<td>C</td>
<td>0.4219</td>
<td>3.675</td>
<td>4.107</td>
<td>3.75</td>
</tr>
</tbody>
</table>

Figure 6.8: Differences in width of spleen showing mean and standard deviation of three groups
Figure 6.9: Differences in width of spleen showing spot typographical errors on data

6.2.3.2 Histological study

For histological study, fixed spleen was cut at 6 µ and stained in Delafield’s hematoxylin and eosin and prepared slides were observed under microscopy (Figure 6.10)
<table>
<thead>
<tr>
<th>Image</th>
<th>Findings</th>
</tr>
</thead>
</table>
| ![Image](normal_spleen_10X.jpg) | **Figure 6.10 (a)**  
Normal Spleen 10X |
| ![Image](normal_spleen_40X.jpg) | **Figure 6.10 (b)**  
Normal spleen 40X |
Figure 6.10 (c)
Cadmium chloride group 10X:
Photograph showing focal depopulation (white arrow), necrosis (blue arrow) & hyperplasia of white matter (yellow arrow)

Figure 6.10 (d)
Cadmium chloride group 40X:
Photograph showing focal depopulation (white arrow), necrosis (blue arrow)
Figure 6.10 (e)
Cadmium Chloride + TU Phytosomal syrup group 10X:
photomicrograph showing normal sinus.

Figure 6.10 (f)
Cadmium Chloride + TU Phytosomal syrup group 40X:
Photomicrograph showing normal sinus
Figure 6.10 (g)
Cadmium chloride + TU Phytosomal syrup group 100X:
Photomicrograph showing normal sinus

Figure: 6.10  Photomicrograph of rat spleen fixed with Bouin’s hematoxylin and eosin

Statistical analysis of the length and width of spleens among different groups revealed that CdCl₂ feeding caused splenomegaly, but when phytosomal syrup of Tecomella undulata was administered along with CdCl₂, the spleen size remained normal. Histology of the spleen revealed that ingestion of CdCl₂ caused congestion of sinuses and hyperplasia. When Phytosomal syrup of Tecomella undulata extract was administered along with CdCl₂ the spleen showed almost normal histology.

6.2.3.3 Hematological study

The hematologic data of control group (group I), cadmium chloride group (group II), and phytosomal syrup (group III) treated wistar rats was given in table 6.10.
6.3 DISCUSSION

When the spleen enlarges, it trap and stores excessive number of blood cells and platelets (hypersplenism), thereby reducing the number of red blood carpusals and platlets in the bloodstream. This process creates a vicious circle: the more cells and platlets the spleen traps, the larger it grows, the more cells and platelets it traps. Eventually, the greatly enlarged spleen also traps normal red blood cells, destroying them along with abnormal ones. In, addition, excessive numbers of blood cells and platelet can clog the spleen, interfering with its function.

The results showed that CdCl$_2$ ingestion occurred splenomegaly and hyperplasia. When Tecomella undulata extract was administered along with Cd in the food, the almost normal (less than 10% enlargement) in spleen was observed as well as no major change in blood profile was observed. This suggests a protective effect of Tecomella undulata extract against splenomegaly.

Same activity but some better results were found when phytosomal syrup of Tecomella undulata was given to wistar rats. When phytosomal syrup of Tecomella undulata was administered along with Cd in the food, no change in the histo-morphology of the spleen was observed as well as no major change in blood profile was observed. Histology of the spleen revealed that CdCl$_2$ ingestion caused congestion of sinuses and hyperplasia. When Tecomella undulata extract as well as phytosomal syrup were administered along with CdCl$_2$ the spleen showed almost normal histology.

Data of Anova table showed that Phytosomal syrup of Tecomella undulata was showed better histopathological improvements than extract of Tecomella undulata in animals study. It may be due to the, extracts when taken orally some constituents may be destroyed in the gastric environment. As standardized extracts are established, poor bioavailability often limits their clinical utility due to above said reasons but Phytosomal syrup is more bioavailable as compared to conventional herbal extracts owing to their enhanced capacity to cross the lipoidal biomembrane and nally reaching the systemic circulation.
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