Figure (51): Western blot expression of IL1-β in control and experimental groups of animals.

(A) L1 L2 L3 L4

IL1-β (35kDa)

(B)

β-actin (42kDa)

5. INVITRO STUDY

5.1 In vitro assay for Cytotoxicity activity (MTT assay).

Arjunolic acid, a triterpenoid saponin, is a major component present in the isolated extracts of the bark of TA. Triterpenoids, an important class of plant secondary metabolites derived from C₃₀ precursors, possess wide range of biological activities, obtained from local market and tested for its protective role against CsA induced functional and structural renal apoptosis.
In the present investigation, the cytotoxicity study of Arjunolic acid was carried out and the results of Arjunolic acid extract at various doses (400, 200, 100, 50, 25, 12.5, 6.25, 3.125 µg/ml) after a week was determined on VERO cells. The cell viability rate of MTT assay was found to be decreased with increased concentration of samples and a plot of concentration versus percent cell viability on the graph produced an approximate linear correlation between them. From the graph, the concentration at which 50% cell viable was determined and the concentration of the extract of Arjunolic acid was found to be 50µg/ml.

In MTT assay, the chloroform methanol extract of Arjunolic acid exhibited significant cytotoxic activity with the concentration value of 50µg/ml. So from this result, extract of Arjunolic acid possesses some cytotoxic effects only at very low concentration. Recent data suggested Diazoxide and cyclosporine A protect primary cholinergic neurons against beta-amyloid (1-42)-induced cytotoxicity (Zeng et al., 2013). Recently reported that antimicrobial, antioxidant and cytotoxic effects of the bark of Terminalia Arjuna have been determined (Shafikur Rahman & Salma Sultana, 2011). It is also been reported that AA protects arsenic induced cytotoxicity in isolated murine hepatocytes (Manna et al., 2007).
Table 10: Cytotoxicity effect of Sample on VERO cell line

<table>
<thead>
<tr>
<th>S. No</th>
<th>Concentration (µg/ml)</th>
<th>Dilutions</th>
<th>Absorbance (O.D)</th>
<th>Cell viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>400</td>
<td>Neat</td>
<td>0.14</td>
<td>24.56</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>1:1</td>
<td>0.19</td>
<td>33.33</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>1:2</td>
<td>0.24</td>
<td>42.1</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>1:4</td>
<td>0.29</td>
<td>50.87</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>1:8</td>
<td>0.37</td>
<td>64.91</td>
</tr>
<tr>
<td>6</td>
<td>12.5</td>
<td>1:16</td>
<td>0.45</td>
<td>78.94</td>
</tr>
<tr>
<td>7</td>
<td>6.25</td>
<td>1:32</td>
<td>0.50</td>
<td>87.71</td>
</tr>
<tr>
<td>8</td>
<td>3.125</td>
<td>1:64</td>
<td>0.53</td>
<td>96.49</td>
</tr>
<tr>
<td>9</td>
<td>Cell control</td>
<td>-</td>
<td>0.57</td>
<td>100</td>
</tr>
</tbody>
</table>
Cytotoxicity effect of Sample on VERO cell line

Figure: 53

Normal VERO Cell line

Toxicity- 400µg/ml

Toxicity- 100µg/ml

Toxicity- 50µg/ml

Toxicity- 25µg/ml
REFERENCES (DISCUSSION)


