CHAPTER 9:
HEPATOPROTECTIVE
ACTIVITY OF COLUMN
CHROMATOGRAPHIC
FRACTIONS (A1KDC AND
EA2KDC)

9.1 Materials and methods
9.2 Results and discussion
9.3 Conclusion
References
The next essential step is finding out of its biological activity of column fractions, A1KDC and EA2KDC. As the fractions isolated from PE and EA extracts and these crude extracts showed a significant (P<0.05) hepatoprotective activity (discussed in chapter 7), therefore, the isolated fractions were tested for the presence of hepato-curative agents which can overcome the hepatotoxic effects in female Swiss albino mice *in vivo*.

**9.1 MATERIALS AND METHODS**

Materials and methods are similar to that discussed in section 7.2 of chapter 7. Five groups of mice were selected, each group having randomly selected 6 mice.

- **Group I**: Control (Pure water 10 ml/kg, oral dose)
- **Group II**: CCl₄: Liquid paraffin (1:1; 2ml/kg, intraperitoneally)
- **Group III**: Silymarin (25mg/kg, oral dose) + CCl₄: Liquid paraffin (1:1; 2ml/kg, intraperitoneally)
- **Group IV**: A1KDC (100 mg/kg, oral dose) + CCl₄: Liquid paraffin (1:1; 2ml/kg, intraperitoneally)
- **Group V**: EA2KDC (100 mg/kg, oral dose) + CCl₄: Liquid paraffin (1:1; 2ml/kg, intraperitoneally)

Measurement of biochemical parameters, histopathological studies and statistical analysis are done as discussed in section 7.2.

**9.2 RESULTS**

**9.2.1 Biochemical Parameters**

The animals treated with CCl₄ exhibited a significant (*p < 0.05*) rise in SGPT, SGOT, ALKP and bilirubin levels when compared to the control group. This were significantly (*p < 0.05*) reduced after treatment with A1KDC and EA2KDC, which were almost similar to that of silymarin (Table 9.1). Graphical representation of the data clearly signifies nearly similar results in case of silymarin and column fractions treated groups (Figure 9.1).
Table 9.1: Effect of column fractions (AlKDC and EA2KDC) on serum biochemical parameters against CCl₄-induced hepatotoxicity in Swiss Albino mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SGPT (U/L)</th>
<th>SGOT (U/L)</th>
<th>ALKP (U/L)</th>
<th>Bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>50.63± 0.30</td>
<td>65.67± 0.50</td>
<td>105.03± 0.17</td>
<td>8.92± 0.23</td>
</tr>
<tr>
<td>Group II</td>
<td>144.33± 0.15 a</td>
<td>215.33± 2.14 a</td>
<td>156.63± 0.22 a</td>
<td>11.6± 0.18 b</td>
</tr>
<tr>
<td>Group III</td>
<td>76.24± 0.78 b</td>
<td>41.32± 0.23 b</td>
<td>76.88± 0.19 b</td>
<td>10.52± 0.18 b</td>
</tr>
<tr>
<td>Group IV</td>
<td>67.08±1.16 b</td>
<td>57.89± 0.50 b</td>
<td>49.67±0.28 b</td>
<td>8.14± 0.13 b</td>
</tr>
<tr>
<td>Group V</td>
<td>60.23±2.1 b</td>
<td>58.78±0.78 b</td>
<td>51.12±0.56 b</td>
<td>9.45± 0.45 b</td>
</tr>
</tbody>
</table>

Values are expressed as mean± standard error (SE); n=6 in each group. aP<0.05 vs control group; bP<0.05 vs CCl₄ treated group.

Figure 9.1: Graph showing serum biochemical parameters (SGPT, SGOT, ALKP and Bilirubin) of Column Fractions (AlKDC and EA2KDC)
9.2.2 Lipid peroxidation

The liver MDA was found to be significantly ($p < 0.01$) higher in the CCl$_4$ treated group than measured in the control group. Treatment with the column fractions decreased the elevated MDA levels. The MDA level for silymarin was also found to be significantly decreased ($p < 0.05$) (Table 9.2).

9.2.3 Total Protein

Total protein level was significantly ($p< 0.05$) reduced in the CCl$_4$ treated group when compared to the control and were significantly elevated ($p < 0.05$) in AIKDC and EA2KDC treated group. These were comparable with that of silymarin-treated group (Table 9.2).

9.2.4 Antioxidant enzymes and Glutathione Levels

The levels of antioxidant enzymes such as CAT and SOD and GSH were decreased significantly ($p< 0.05$) after CCl$_4$ treatment and were significantly ($p < 0.05$) elevated in AIKDC and EA2KDC treated group (Table 9.2).
Table 9.2: Effect of column fractions (A1KDC and EA2KDC) on liver malondialdehyde (MDA), total protein, glutathione (GSH) and antioxidant enzymes against CCl₄-induced hepatotoxicity in Swiss Albino mice.

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>MDA (μmol/g tissue)</th>
<th>Protein (μg/ml)</th>
<th>GSH (μ mol of GSH/ g wet tissue)</th>
<th>SOD (units/mg liver protein)</th>
<th>CAT (units/mg liver protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>47.2±0.78</td>
<td>24.56±1.12</td>
<td>34.33±0.32</td>
<td>45.23±0.78</td>
<td>43.3±0.22</td>
</tr>
<tr>
<td>II</td>
<td>134.1±1.8</td>
<td>9.04±0.89</td>
<td>12.45±1.12</td>
<td>22.78±0.32</td>
<td>25.74±0.38</td>
</tr>
<tr>
<td>III</td>
<td>50.21±0.32</td>
<td>14.56±1.12</td>
<td>34.45±0.32</td>
<td>46.32±0.32</td>
<td>44.72±2.1</td>
</tr>
<tr>
<td>IV</td>
<td>56.42±0.78</td>
<td>19.32±2.08</td>
<td>46.19±0.22</td>
<td>57.08±0.78</td>
<td>58.62±1.12</td>
</tr>
<tr>
<td>V</td>
<td>44.23±0.33</td>
<td>18.78±0.78</td>
<td>44.45±1.12</td>
<td>46.21±0.33</td>
<td>58.12±0.38</td>
</tr>
</tbody>
</table>

Values are expressed as mean± standard error (SE); n=6 in each group. *P<0.05 vs control group; †P<0.05 vs CCl₄ treated group.

Figure 9.2: Graph showing biochemical parameters (CAT, SOD, GSH, Protein content and MDA) of Column Fractions (A1KDC and EA2KDC).
9.2.5 Histopathology

The liver section of mice treated with 100 mg/kg bodyweight, oral dose of A1KDC and EA2KDC followed by CCl₄ intoxication (Fig 9.3 and 9.4) showed less vacuole formation and absence of necrosis and overall less visible changes observed were comparable with standard silymarin, supplementing the protective effect of the test drug and the standard hepatoprotective drug.

Figure 9.3: Transmission Electromicrograph of mice liver treated with CCl₄ + Column Fraction A1KDC (100mg/kg).

Figure 9.4: Transmission Electromicrograph of mice liver treated with CCl₄ + Column Fraction EA2KDC (100mg/kg).
9.2.6 Body weight

Body weight of mice gradually increased in case of control group and decreased in CCl4 treated group. Increase in body weight in silymarin, A1KDC and EA2KDC treated groups reveal the presence of hepatocurative effects of these groups.

Table 9.3:- Effect of column fractions (A1KDC and EA2KDC) on body weight of Swiss Albino mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body Weight of Mice in Grams</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st Day</td>
</tr>
<tr>
<td>Group I</td>
<td>25.33± 2.2</td>
</tr>
<tr>
<td>Group II</td>
<td>26.4± 2.4</td>
</tr>
<tr>
<td>Group III</td>
<td>25.2± 3.2</td>
</tr>
<tr>
<td>Group IV</td>
<td>26.5± 0.98 b</td>
</tr>
<tr>
<td>Group V</td>
<td>27.34± 1.1 b</td>
</tr>
</tbody>
</table>

Values are expressed as mean± standard error (SE); n=6 in each group. *P<0.05 vs control group; bP<0.05 vs CCl4 treated group.

The hepatoprotective test results of the column fractions, A1KDC (100 mg/ kg) and EA2KDC (100 mg/ kg) was compared with that of PE (200 mg/ kg) and EA (200 mg/ kg) extracts as these two fractions were isolated from PE and EA crude extracts respectively. SGPT levels of A1KDC, EA2KDC, PE and EA treated groups were 67.08, 60.23, 66.34 and 121.05 U/L respectively showing that EA2KDC had more actively reduced the enzyme level than other treated groups. In case of SGOT, PE extract treated group represented least enzyme level (55.87 U/L) followed by A1KDC (57.89 U/L), EA2KDC (58.78 U/L) and EA (65.76 U/L). Maximum reduced level of ALKP was obtained in
case of EA2KDC (51.12 U/L) and that of bilirubin in case of PE crude extract (6.13 mg/ dl).

Results of MDA test showed that EA2KDC efficiently lowered (44.23 μmol/ g tissue) the elevated MDA level amongst these four test groups. On the other hand, maximum elevation of antioxidant enzymes were observed in PE treated group in case of GSH (74.34 μ mols of GSH/ g wet tissue), EA treated group in case of SOD (64.39 units/ mg liver proteins) and PE treated group again in case of CAT (67.11 units/ mg liver proteins). The results suggest that the observations in case of column fractions and their crude extracts were nearly similar and comparable with the standard drug silymarin.

9.3 CONCLUSION

In conclusion it can be said that the fractions, A1KDC and EA2KDC have significant (P< 0.05) hepatoprotective activity as per in vivo test performed in Swiss Albino mice. So, attention should be given in structural derivation of these compounds in pure form and clinical trials to confirm their bioactive principles.

9.4 REFERENCES


