HEPATOPROTECTIVE ACTIVITY OF METHANOLIC EXTRACT OF CLEOME GYNANDRA LEAVES AGAINST CCL4 INDUCED HEPATOTOXICITY IN RATS

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Abstract
The present study deals with the investigation of the hepatoprotective effect of methanolic leaf extract of Cleome gynandra. Biochemical parameters like Serum Glutamate Oxaloacetate transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), alkaline phosphatase (ALP), serum triglycerides (TG), Total bilirubin (TB), Total protein (TP), Albumin (AB), Prothrombin Time (PT) and Total Cholesterol (TC) were measured. The administration of single dose of 1.25 ml/kg of carbontetrachloride cause an significant increase in the serum levels of SGPT, SGOT, ALP, TB, TC, TG and significant decrease in TP, AB, PT biochemical parameters whereas group of rats pretreated orally with silymarin (10 mg/kg) and methanolic extracts (200 and 400 mg/kg) of Cleome gynandra for five consecutive days showed significant dose dependent reversal (p<0.05) in the levels of all estimated biochemical parameters. The overall experimental results suggests that the biologically active phytoconstituents such as flavonoids, tannins, terpenoids present in the methanolic leaf extract of Cleome gynandra, may be responsible for the significant hepatoprotective activity and the results justify the use of Cleome gynandra as a hepatoprotective agent.

Keywords: Cleome gynandra, CCl4, Silymarin, Hepatoprotective, Biochemical parameters.

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INTRODUCTION

The liver is responsible for metabolism and detoxification of the most of components that enter the body. Liver disease is an important cause of morbidity and mortality in the United States, affecting persons of all ages, but most frequently individuals in the productive years of life, between the ages of 40 and 60 years. They are mainly caused by chemicals and some drugs when taken in very high doses. Despite advances in modern medicine, there is no effective drug available that stimulates liver function, offer protection to the liver from damage or help to regenerate hepatic cells. The available synthetic drugs to treat liver disorders in this condition also cause further damage to the liver. Hence, there is urgent need for effective drugs to replace/supplement those in current use. The plant kingdom is undoubtedly valuable as a source of new medicinal agents. Carbon tetrachloride (CCl4) is a highly toxic chemical agent, the most famous drug used to induce liver damage experimentally. The toxic effect of CCl4 is attributed to trichloromethyl radical produced during oxidative stress. Cleome gynandra is an annual herb belongs to the family Capparidaceae. It is popular indigenous system of medicine like Ayurveda, Siddha, Unani and Homeopathy and used traditionally as anthelmintic, anti-inflammatory, anti-microbial and antineoplastic. Preliminary phytochemical screening of methanolic extract of Cleome gynandra, showed the presence tannins, saponins, flavonoids, steroids and terpenoids. Literature revealed that plant containing flavonoids were responsible for hepatoprotective activity and the Cleome gynandra also having these constituent so it was hypothesized that it may also be able to protect the liver. Keeping this view the present study scientifically validated the traditional use of Cleome gynandra for liver disorders.

MATERIAL AND METHODS

Plant Materials

The leaves of Cleome gynandra were collected from the native species growing in Tirupathi region, Andhra Pradesh, India. The leaves have been identified taxonomically and authenticated by Dr. S. Madhava chetty, Associate Professor, Department of Botany, Sri Venkateswara University, Tirupathi, Andhra Pradesh, India.

Preparation of plant extract

The plant material was cleaned, shade dried and powdered mechanically. The powdered leaves of Cleome gynandra were subjected to soxhlet extraction with methanol. The extract was concentrated by vacuum distillation. The extract was subjected to phytochemical screening.
Experimental animals
Male Wistar Albino rats (150-200 g) were procured from Albino Research Center. They were randomly housed in standard polypropylene cages and maintained under the standard conditions: room temperature (25 ± 3)°C, humidity 45% - 55%, 12/12 h light/dark cycle. They were fed with commercially available pellet diet obtained from Amruth foods, Pranav Agro Industries, Sangli, India and water was allowed ad libitum. The animals were acclimatized to the laboratory conditions minimum one week prior to the behavioral experiments.
Animals used in this study were treated and cared for in accordance with the guidelines recommended by CPCSEA. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) of MRIPS, Secunderabad (Letter no 25/418/2009-AWD) with CPCSEA (Reg.No: 1662/PO/a/CPCSEA, 2013).
Hepatotoxicity was induced by single intra peritoneal injection of carbon tetrachloride: olive oil in 1:1 ratio. Animals were divided into five groups consisting of six animals in each group. First group of animals were received only vehicle (1% CMC, p.o.) once daily for 7 days and served as normal group.
Second group of animals received vehicle (1% CMC, p.o.) for 1-7 days and CCl₄ (1.25ml/kg) on 7th day and served as disease control group.
Third group of animals received silymarin (10mg/kg) p.o once daily for 7 days and CCl₄ (1.25ml/kg, i.p.) on 7th day and served as standard group, fourth and fifth groups received MECG (200 & 400 mg/kg p.o) once daily for 7 days and CCl₄ (1.25 ml/kg, i.p.) on day-7.
Estimation of biochemical parameters
After 48 h, following CCl₄ administration, blood samples were collected from the retro orbital plexus and serum was separated for analyzing SGOT [12], SGPT [13], ALP [14], serum total bilirubin [15], serum triglycerides [16], serum total protein [17], serum cholesterol [18], serum triglycerides [19] and prothrombin time [20].
Statistical analysis
All the data were expressed as Mean ± Standard Error (SEM). Data obtained from various groups was subjected to one-way analysis of variance (ANOVA) followed by Dunnett’s t-test. P< 0.05 was considered to be statistically significant.
RESULTS AND DISCUSSION

Biochemical parameters

The administration of single dose of CCl₄ (1.25 ml/kg, p.o.) raised the serum levels SGOT, SGPT, ALP, total bilirubin, triglycerides, cholesterol, prothrombin-time and decreased the serum albumin and total protein levels significantly (p<0.05) compared to normal group animals. All the estimated biochemical parameter levels were reversed significantly (p<0.05) in the rats treated with Cleome gynandra leaf extract was depicted in Table 1 and Table 2.

Table 1: Effect of methanolic leaf extract of Cleome gynandra on biochemical parameters

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>ALP (U/L)</th>
<th>Total Bilirubin (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>36.56±1.50</td>
<td>47.96±1.89</td>
<td>73.16±3.53</td>
<td>0.53±0.09</td>
<td>67.59±2.81</td>
</tr>
<tr>
<td>CCl₄</td>
<td>130.72±4.22</td>
<td>149.71±4.57</td>
<td>211.26±5.19</td>
<td>1.87±0.12</td>
<td>132.62±3.50</td>
</tr>
<tr>
<td>Silymarin</td>
<td>56.52±2.59</td>
<td>62.49±3.38</td>
<td>111.40±3.01</td>
<td>0.68±0.09</td>
<td>75.61±2.76</td>
</tr>
<tr>
<td>MECG (200 mg/kg)</td>
<td>72.96±3.05</td>
<td>89.88±2.46</td>
<td>149.01±4.19</td>
<td>1.22±0.08</td>
<td>109.11±4.97</td>
</tr>
<tr>
<td>MECG (400 mg/kg)</td>
<td>63.74±2.79</td>
<td>65.75±2.76</td>
<td>121.04±4.07</td>
<td>0.90±0.07</td>
<td>84.62±1.86</td>
</tr>
</tbody>
</table>

Values are expressed as mean ±SEM for six animals; MECG=Methanolic Extract of Cleome gynandra
a= p<0.05 as compared to normal control; b= p<0.05 as compared to CCl₄ treated group

Table 2: Effect of methanolic leaf extract of Cleome gynandra on biochemical parameters

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cholesterol (mg/dl)</th>
<th>Albumin (mg/dl)</th>
<th>Total protein (mg/dl)</th>
<th>Prothrombin Time (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>48.89±2.92</td>
<td>3.81±0.11</td>
<td>6.07±0.19</td>
<td>17.79±0.50</td>
</tr>
<tr>
<td>CCl₄</td>
<td>81.04±2.12</td>
<td>1.37±0.10</td>
<td>2.62±0.16</td>
<td>41.19±1.24</td>
</tr>
<tr>
<td>Silymarin</td>
<td>55.19±2.37</td>
<td>3.28±0.09</td>
<td>5.42±0.14</td>
<td>21.04±1.20</td>
</tr>
<tr>
<td>MECG (200 mg/kg)</td>
<td>64.09±2.34</td>
<td>2.55±0.11</td>
<td>4.14±0.14</td>
<td>37.99±0.94</td>
</tr>
<tr>
<td>MECG (400 mg/kg)</td>
<td>58.59±2.34</td>
<td>3.13±0.10</td>
<td>4.93±0.21</td>
<td>30.56±0.69</td>
</tr>
</tbody>
</table>

Values are expressed as mean ±SEM for six animals; MECG=Methanolic Extract of Cleome gynandra
a= p<0.05 as compared to normal control; b= p<0.05 as compared to CCl₄ treated group

CONCLUSION

Carbon tetrachloride is used as hepatotoxic agent to study the hepato-curative agent in plants and other compounds [21]. In the present study, administration of single dose of CCl₄ successfully
induced the liver damage in the rats. Altered levels of biochemical parameters like increased serum SGOT, SGPT, ALP, total bilirubin, total cholesterol and triglycerides and reductions in total protein, albumin and prothrombin time indicates liver malfunction. Increased total bilirubin, total cholesterol, triglyceride concentrations and in the activities of liver enzymes such as SGOT, SGPT, ALP on induction with CCl4 were ameliorated at 400 mg/kg of MECG. Similarly reductions observed in albumin, total protein and prothrombin time were ameliorated only at 400 mg/kg of MECG. This demonstrates the dose dependent hepatoprotective effect of methanolic leaf extract of *Cleome gynandra*. This hepatoprotective effect can be attributed to high content of flavonoids, tannins and terpenoids [22]. Earlier reports find that CCl4 treatment generates free radicals that trigger a cascade of events resulting in hepatic damage [23] and natural antioxidants like flavonoids, tannins and terpenoids may counter the effects of the free radicals produced by CCl4 which may contribute to the hepatoprotective effect.

REFERENCES


23. Obi FO, Unsenu IA and Osayande JO: Prevention of CCl4 induced hepatotoxicity in the rat by H. rosasinensis anthocyanin extract administered in ethanol. Toxical 1998; 131.93-98.