Experimental researches on preclinical toxicity studies of ethanol leaf extract of *Cordia sebestena*

Sarathchandiran I*1 and Gnanavel M* 2

1Gokula Krishna College of Pharmacy, Sullurupet– 524 121, Nellore District, Andhra Pradesh, India
2Research Scholar, PRIST UNIVERSITY, Vallam, Thanjavur – 613 403, Tamil Nadu, India

ABSTRACT

The acute and sub-acute toxicities of the ethanol leaf extract of *Cordia sebestena* was investigated. For the acute toxicity study, 50-5000 mg/kg of the ethanol leaf extract were administered to rats and obvious toxic symptoms and mortality 24 hours post-administration of the extract were determined. The median lethal dose (LD<sub>50</sub>) of the extract was determined. In the sub-acute study, 200-400 mg/kg of the extract were administered daily for 14 days. The food and water consumption, body weight changes, as well as haematological and biochemical parameters were determined periodically. The estimated LD<sub>50</sub> of the extract was 4000 mg/kg. There was no mortality during the period of study. The effects on haemoglobin concentration, PCV, RBC and WBC counts were non significant. The extract caused non significant levels in serum liver enzymes, AST, ALP and ALT. Histopathological report also normal which supported the non toxic effect of extract. These results suggest that the leaf extract of *Cordia sebestena* is safe for the treatment of Diabetes mellitus.

Keywords: *Cordia sebestena*; toxicity study; median lethal dose study; liver function test

INTRODUCTION

Natural products are the cornerstone of health care delivery, especially in resource poor settings. Present estimates indicate that about eighty percent of the world’s population relies on traditional medicine for health care delivery (Farnsworth, et al 1985, Akah 2008, Appidi et al 2008). This should be encouraged especially in countries where access to the conventional treatment is inadequate, in as much as efficacy and safety are assured (WHO, 1980). A number of studies have reported the toxic effects of herbal medicines (Kalplowitz 1997, Calixto 2000, Jaouad et al, 2004, Ta-ziebou et al, 2008). Studies of medicinal plants using scientific approaches showed that various biological components of medicinal plants exhibit a variety of properties and can be used to treat various ailments.

*Cordia sebestena* (L.) (Boraginaceae Family) is commonly known as the Geiger tree. Hawaiians refer to the plant as Kou Haole though, which roughly translates to “foreign plant” (Abbott, 1992). Recent archaeological evidence indicates that the plant is actually indigenous to the islands (Burney et al., 2001). Regardless of its origin, the plant has a long history of use in Hawaiian culture. The plant’s large dark green leaves have often been used to dye kappa, or wood cloth, that was used for both clothing and bedding. C. sebestena’s dark orange flowers are typically used to make Leis. The plant is best known in the Hawaiian Island for its wood, which due to their lightweight, durable and easily workable nature, are used for many traditional items ranging from canoes to food vessels. The plant can grow up to 25 feet tall in tropical and sub-tropical areas where it is widely distributed due to its extensive use in landscaping.

The present study was designed to evaluate the acute and sub-acute toxicity studies of ethanolic extract of *Cordia sebestena*.

MATERIALS AND METHODS

Experimental animals

Male Wistar rats of body wt. 180–200 g were obtained from central Animal House, Institute name. The animals were fed on standard pellet diet (Hindustan Lever, Mumbai, India) and water ad libitum. The rats used in the present study were maintained in accordance with guidelines of the CPCSEA, India and the study approved by the ethical committee (approval number).

Preparation of plant extract

Leaf of *Cordia sebestina* was collected from the Tirumala forest, Tirupati in June 2011. Their botanical identities were authenticated by Dr. K. Madhava Chetty, Department of Botany, Sri Venateswara University, Tirupati. The shade dried plant were powdered to get a
course granule. About 250 g of dried powder was extracted with 70% ethanol by continuous hot percolation, using soxhlet apparatus, (before that the crude powders were extracted with various solvents in increasing polarity and the antioxidant potential principles like flavonoids were identified in ethanolic extract). The resulted dark – brown extract was concentrated up to 100 ml on Rota vapour under reduced pressure. The concentrated crude extracts were lyophilized in order to powder and used for the study.

**Phytochemical screening**

The alcoholic extract obtained were subjected to preliminary phytochemical screening, to identify the chemical constituents. The methods of analysis employed were those described by Harbone & Baxter, 1993.

**Acute toxicity study**

The acute toxicity and lethality of the EECS was determined by using the method described by Lorke. Briefly, 9 rats were randomly divided into three groups (n=3), and were orally administered with 50, 100, and 1000 mg/kg of the EECS, respectively. They were observed for 24 h for death. Since no death was recorded, 1500 mg/kg, 3000 mg/kg and 5000 mg/kg of the extracts were administered to a fresh batch of animals at one animal per dose and the number of deaths in 24 h was

### Table 1: Phytochemical screening of different leaf extract of *Cordia sebestina*

<table>
<thead>
<tr>
<th>Chemical constituents</th>
<th>Tests</th>
<th>Ethanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Dragendorff’s test</td>
<td>–ive</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Molisch’s test</td>
<td>–ive</td>
</tr>
<tr>
<td>Saponins</td>
<td>Foam test</td>
<td>–ive</td>
</tr>
<tr>
<td>Phenols</td>
<td>Lead acetate test</td>
<td>–ive</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Shinoda test</td>
<td>+ive</td>
</tr>
<tr>
<td>Tannins</td>
<td>Gelatin test</td>
<td>–ive</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>Libermann Burchard test</td>
<td>+ive</td>
</tr>
<tr>
<td>Triterpenes</td>
<td>Salkowski test</td>
<td>+ive</td>
</tr>
</tbody>
</table>

+ive Positive (Present); –ive Negative (Absent)

### Table 2: Effect of ethanolic extract of *Cordia sebestena* on hemoglobin, PCV, RBC and WBC counts

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Hemoglobin g/dl</th>
<th>PCV %</th>
<th>RBC (x10^12/L)</th>
<th>WBC (x10^9/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre value</td>
<td>Post value</td>
<td>Pre value</td>
<td>Post value</td>
</tr>
<tr>
<td>Control Saline 10 ml/kg</td>
<td>10.3±0.32</td>
<td>10.9±1.4</td>
<td>33±2.1</td>
<td>33.9±0.14</td>
</tr>
<tr>
<td>EECS 200 mg/kg</td>
<td>10.6±0.57</td>
<td>11.4±0.22</td>
<td>35±1.5</td>
<td>35.2±0.15</td>
</tr>
<tr>
<td>EECS 400 mg/kg</td>
<td>11.1±0.21</td>
<td>11.7±0.89</td>
<td>36±1.4</td>
<td>35.8±0.17</td>
</tr>
</tbody>
</table>

All values expressed as mean±SEM

![Figure 1: Effect of ethanolic extract of *Cordia sebestena* on hemoglobin, PCV, RBC and WBC counts](image-url)
Sub-acute toxicological studies

Fifteen rats were randomly divided into three groups of 5 rats each. Group 1 animals served as control and received normal saline (10 ml/kg oral). The remaining groups II, and III received 200 and 400 mg/kg of the extract daily for 14 days. Water and food consumption patterns of the rats were determined every 24 hours. After last day, treatment blood samples were collected through cardiac puncture and were used in the estimation of haemoglobin concentration (Hb), packed cell volume (PCV), red blood cell (RBC) and white blood cell (WBC). Serum was separated by centrifuging at 2,500 rpm for 10 minutes and was then used in the analysis of biochemical hepatic markers – aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) by using respective kits. Kidney, liver and heart were isolated and used for histopathological studies.

Statistical analysis

The data for various biochemical parameters were analyzed using analysis of variance (ANOVA) and the group means were compared by dunnet’s test (GraphPad Version 3.06). Values were considered statistically significant when at P < 0.05.

RESULTS AND DISCUSSION

Phytochemical screening

Results of phytochemical screening of both the extracts were revealed that the presence of flavonoids, terpenes, phytosterols. Presences of phytoconstituents were tabulated in Table 1.

Acute toxicity test

The acute lethality and toxicity (LD50) test of the extract showed estimated LD50 values of 4000 mg/kg. At higher doses, anorexia, hyperventilation and diarrhea were observed. From this LD50 value 1/10th and 1/20th doses were selected for further studies.

Sub-acute toxicity study

There was no significant difference in food, water intake and body weight of animals treated with EECS in two different dose when compared to control. Hematological studies revealed a non significant level of he-

### Table 3: Effect of ethanolic extract of *Cordia sebestena* on AST, ALT and ALP

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre value</td>
<td>Post value</td>
<td>Pre value</td>
</tr>
<tr>
<td>Control Saline 10 ml/kg</td>
<td>78±2.6</td>
<td>83±1.6</td>
<td>54.4±1.0</td>
</tr>
<tr>
<td>EECS 200 mg/kg</td>
<td>79±3.7</td>
<td>81±1.2</td>
<td>56.3±1.3</td>
</tr>
<tr>
<td>EECS 400 mg/kg</td>
<td>76±5.6</td>
<td>80±2.2</td>
<td>57.6±1.5</td>
</tr>
</tbody>
</table>

All values expressed as mean±SEM
moglobin, PCV, RBC and WBC concentration when compared to control rats (Table 2 and Figure 1). Biochemical studies showed non-significant levels of serum AST, ALT and ALP when compared to control rats (Table 3 and Figure 2). No mortality was recorded throughout the duration of the sub-acute toxicity studies.

Effect of extract on heart, kidney and liver

There was no significant difference in the weight of kidney, liver and heart of EECS treated and control rats. Histopathological examination of kidney, liver and heart of the control rats and EECS treated rats (200 and 400 mg/kg), showed normal cellular architecture without any necrotic and inflammatory changes. Figure 3a-Figure 3c shows normal myocardial fibres with clear nuclei. Figure 3d-Figure 3f shows normal architecture of kidney with normal glomeruli and peritubular structure. Figure 3g-i normal hepatocytes with clear central vein.

CONCLUSION

In conclusion, these results provide evidence for the safety profile of the ethanol leaf extract of Cordia sebestena thus supporting the validity for its use in the treatment of Diabetes mellitus.

REFERENCES


Jaouad, EH, Israilli ZH, Lyoussi B. 2004 Acute toxicity and chronic toxicological studies of Ajugaiva in experimental animals. J. Ethnopharmacol. 91:43-50
