Hepatoprotective and antioxidant properties of *Suaeda maritima* (L.) Dumort ethanolic extract on concanavalin-A induced hepatotoxicity in rats

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Received 12 May 2010; Revised 16 March 2011

Hepatoprotective and antioxidant properties of *Suaeda maritima* (L.) Dumort on concanavalin-A induced stress in Wistar albino rats have been reported. Rats were administered with ethanolic extract of *Suaeda maritima* at the concentration of 75, 150 and 300 mg/kg of body wt. for 9 days and concanavalin-A was administrated (iv) 12 mg/kg on 9th day. Rats in concanavalin-A administered group showed elevated levels of AST, ALT, ALP and bilirubin. Pretreatment of rats with ethanolic extract (300 mg/kg) significantly reduced these serum parameters compared to concavalin-A administered group. Histopathological examination of liver sections showed that, normal liver architecture was disturbed by hepatotoxin intoxication. The extract treated group and silymarin treated group retained the normal cell architecture, although less visible changes were observed. Preliminary phytochemical analysis showed the presence of triterpenoids and may be responsible for the hepatoprotective activity. The LD$_{50}$ was calculated as 3 g/kg of the body weight. IC$_{50}$ values of hydroxyl (52.21±1.32 µg/ml) and nitric oxide radicals (09.14±0.94 µg/ml) scavenging results showed comparable activity with vitamin-C. Results of this study may be useful for the development of herbal medicine from *Suaeda maritima* for the treatment of hepatitis.

**Keywords**: Acute toxicity, Concanavalin-A, Hepatoprotective, Salt marsh, *Suaeda maritima*

*Suaeda maritima* (L.) Dumort, belonging to Chenopodiacea family is a salt marsh mangrove herb similar to *Suaeda monoica* in appearance. The herb prefers neutral and basic (alkaline) moist soils and can grow in very alkaline and saline soils. Locally it is called as *umiri* in Pichavaram and *mattamiri* in Muthupet, Tamil Nadu, India. The raw or cooked young leaves has a pleasant salty flavour and are often mixed with other vegetables to reduce their saltiness. The young shoots are pickled in vinegar and eaten on their own or used as a relish. Traditionally, the leaf from *Suaeda maritima* has been used as a medicine for hepatitis and reported to have antiviral activity due to the presence of triterpenoids, sterols. Modern medicines have little to offer for alleviation of hepatic disease and it is chiefly the plant based preparation which is employed for the treatment of liver disorders. But there are not much drugs available for the treatment of liver disorders. It has been reported that, several chemical constitutes of plant origin were evaluated for its possible hepatoprotective activity against chemical induced liver damage in experimental animals. The present study was aimed to evaluate hepatoprotective effect of ethanolic extract of *Suaeda maritima* leaves using concanavalin-A induced liver injury model in Wistar rats.

**Materials and Methods**

*Extraction*—The fresh elder leaves of *Suaeda maritima* were collected from Karangadu (Lat. 9° 38’ N and Lon. 78° 38’ E) mangrove forest, Ramnad district, Tamil Nadu, India and their identity was confirmed by following the standard monograph. The specimen sample was also authenticated by Prof. K. Kathiresan, Centre of Advanced Study in Marine Biology, Annamalai University, Porto Novo, Tamil Nadu, India. The voucher specimen (AUOCAS002) was also deposited at the School of Marine Sciences, Department of Oceanography and Coastal Area Studies, Alagappa University, Thondi Campus, Thondi, India. The leaves were washed thrice with distilled water to remove the contaminants and air dried in shade. Coarsely powdered sample (500 g) was defatted with petroleum ether (60-80°C) and then

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extracted with 1L of 95% (V/V) ethanol and water mixture by percolation method. The extract was concentrated under vacuum to the solvent free residues. The percentage yield of extract was 52.64% (w/w). Analysis of phytochemical constituents was carried out by standard qualitative tests17. In vitro antioxidant assay—Determination of DPPH assay18, hydroxyl radical scavenging assay19, nitric oxide radical scavenging assay20 and superoxide radical scavenging assay21 were performed with various concentrations (1.9 to 500 µg/ml) of S. maritima leaf extract and various concentrations of (1.9 to 500 µg/ml) vitamin C (positive control). The IC50 values were calculated using linear regression with Statplas pro 2009 software package. Treatments—Male Wistar albino rats (150-200 g) were maintained under standard conditions (23±2°C, relative humidity 55 ± 10% and 12:12 h LD cycle) and allowed free accesses to food (Sai Durga Feeds and Foods, Bangalore, India) and water. Experimental protocols were approved by Institutional Animal Ethics Committee, Alagappa University, Tamil Nadu, India. For the determination of median lethal dose (LD50) animals (9) were kept fasting for overnight providing only water, after which the extracts were administrated orally at different doses of 250-5000 mg/kg and all the rats were observed for the physical signs of toxicity for 14 days. If the mortality was observed in 6 out of 9 animals, then the dose administrated was assigned as toxic dose. If mortality was observed in 3 animals, then the same dose was repeated again to confirm the toxic dose. One tenth of the maximum dose of the extract tested for acute toxicity was selected for evaluation of hepatoprotective activity (1:1) and kept as positive control group.

Animals of group IV, V and VI were treated with ethanolic extracts of 75, 150 and 300 mg/kg body wt. were administrated through oral gavage for 9 days + single dose of (12 mg/kg body wt., iv) concanavalin-A on 9th day with liquid paraffin (1:1) and kept as treatment groups.

Analysis of clinical parameters—On the 10th day all the animals were anesthetized with ether. Blood samples were collected from jugular vein. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 600 × g for 15 min and analyzed for AST, ALT, ALP and bilirubin. Blood standard commercial kit (CML-Biotech Pvt. Ltd, India) were used to measure the level of AST, ALT, ALP and Bilirubin. All the determinations were carried out by an auto analyzer of Merck make (300TX, E-Merck-Micro Labs, Mumbai). The hepatoprotective activity (% H), was calculated as follows:

\[ 1 - \frac{(T - V)}{(C - V)} \times 100 \]

Where T is mean value of drug and concanavalin-A, C mean value of concanavalin-A alone and V is the mean value of normal control animals. Values are expressed as mean ± SD which for biochemical parameters statistically using one way ANOVA for comparison with control group and concanavalin-A treated group. P<0.05 was considered as significant.

Histopathological studies—For the histopathological study, the liver of 6 animals from each group were immediately removed after autopsy and the tissues were fixed in bouin’s solution for 12 h, then embedded in paraffin using conventional methods24 and cut in to 5 µm thick sections and stained using haematoxylin-eosin dye and finally mounted in diphenyl xylene. The sections were observed under microscope for histopathological changes. Histological damage was expressed using the following score system: ∅ absent; ↓ few; + mild; ++ moderate; +++ severe; ++++ extremely severe (the liver of 6 animals in every group were examined with 10 different microscopic fields).

Results and Discussion
In Indian system of medicine, certain herbs are claimed to provide relief against liver disorders. The
claimed therapeutic reputation has to be verified in a scientific manner. In the present study one such extract of *Suaeda maritima* was taken for the hepatoprotective study. Preliminary phytochemical screening of the ethanolic extract of the leaf *Suaeda maritima* showed the presence of reducing sugars, steroids, and triterpenes. In the toxicity study, no mortality occurred throughout the experiment with the doses of plant extract and silymarin treated group. The ethanolic extract did not show any mortality up to the level of 3000 mg/kg and were considered as safe. Single dose of concanavalin-A is sufficient for the development of liver lesions. Concanavalin-A induced hepatitis is both T-cell and macrophage dependent. The precise mechanism(s) by which T-cells and macrophages exert their hepatogenic potential is not known. Because a massive release of macrophage and T-cell derived cytokines IL-2, IL-1, IL-6, tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ) and granulocyte macrophage colony stimulating factor (GM-CSF) occurs with different kinetics in response to concanavalin-A, a role has been envisaged for these cytokines in the development of the hepatic lesions. It is evident that, the concanavalin-A produced liver inflammation due to the severe hepatic necrosis. It is known that an increasing the enzymatic activity of ALP and AST in the serum directly reflects the major permeability of cell membrane. An increase in AST and ALT, a hepatospecific enzyme that is principally found in the cytoplasm in rats following the administration of CCl₄ is attributed to the increased release of the enzymes from damaged liver parenchymal cells. Similar results of ACP and ALP were found to increase in hepatotoxin animals.

In the present study concanavalin-A administrated group showed significantly increased levels of AST, ALT, ALP (Fig. 1), direct and indirect bilirubin (Fig. 2). Amino transferase is an important class of enzymes linking carbohydrate and amino acid metabolism there by clearly establishing the relationship between the intermediates of the citric acid and amino acids. Alanine amino transferase and aspartate amino transferase are well known diagnostic indicators of liver diseases. In case of liver damage with hepatocellular lesions and parenchymal cell necrosis, these enzymes are released from the damaged tissue into the blood stream. The decrease in alanine amino transferase activity is usually accompanied by lowering in the activity of aspartate amino transferase, in the present study the activities of aspartate and amino transferase enzymes were maintained at near normal level in the oral administrated group of plant extract as compared with the hepatotoxin group. Alkaline phosphatase activity on endothelial cell surface is responsible for the conversion of adenosine nucleotide to adenosine, a potent vasodilator and anti inflammatory mediator that results from injury. So, following, accumulation of IL-6 can leads to the production of adenosine to alkaline phosphatase, this may be the reason for the increment in ALP in hepatotoxin group. Bilirubin is a major breakdown product that results from the destruction of RBC which is removed from the blood by the liver through conjugation and secreted into bile usually becomes elevated as a result of decreased uptake by the liver, decreased conjugation, decrease secretion from the liver or blockage of the bile ducts that is caused in liver damage. The oral administration of plant extract and (positive drug)
silymarin 100 mg/kg were significantly reduced the level of marker enzymes. The hepatoprotective activity of different doses was also observed and maximum activity of 80.90% was noticed with 300 mg/kg of the plant extract (Table 1).

Histopathological scores (Table 2) showing the severity level of fatty changes and hydrophic changes in concanavalin-A treated rats but, mild level of fatty changes were observed with silymarin and extract (300 mg/kg) treated rats, moreover focal necrosis were observed with severe level in concanavalin-A treated rats but few level of necrosis were observed with extract and silymarin treated rats. Histopathological examination of liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoids space and proper central vein (Fig. 3). Whereas concanavalin-A induced rats showed distorted sinusoids and hepatic necrosis (Fig. 3b). Rats pretreated with silymarin (100 mg/kg) and ethanolic extract (300 mg/kg) followed by concanavalin-A hepatotoxicity showed a sign of protection as it was evident from the reduction of necrosis and normalization of sinusoidal structure (Figs 3c-f).

Results of DPPH assay, hydroxyl radical scavenging activity, nitric oxide radical scavenging activity and superoxide radical scavenging assay of plant extract and vitamin C were represented in Table 3. It revealed that, hydroxyl radical scavenging (52.21 ± 1.32 µg/ml) and nitric oxide scavenging (09.14 ± 0.94 µg/ml) activity showed comparable activity with the standard vitamin C (44.24 ± 1.50 µg/ml and 4.98 ± 1.28 µg/ml). Previously, it was reported that, reactive oxygen species play a major role in Concanavalin-A induced hepatitis through secondary immune mediated liver damage and the hepatoprotective effect of *S. maritima* leaf extract might be due to the presence of antioxidant properties.

It is concluded that, administration of concanavalin-A resulted in a significant increase in the AST, ALP, ALT and bilirubin level and these changes in the marker levels reflected in hepatic structural integrity. Pretreatment with alcoholic extract of *Suaeda maritima* attenuated the elevated level of serum markers. Normalization of serum markers by ethanolic extracts suggested that, hepatocytes are conditioned to protect the membrane integrity against concanavalin-A induced leakage of marker enzymes into the circulation. Elevated level of bilirubin indicates the hepatotoxicity. The normalization of the blood bilirubin extract in pre-treated rats further indicated the protective nature of the extract on hepatic cells. Histopathological examination of liver sections revealed that, the normal liver architecture was disturbed by hepatotoxin intoxication. Sections obtained from the plant extract and silymarin groups showed normal cell architecture, although less visible changes were observed which further corroborate the hepatoprotective activity. The hepatoprotective activity of *Suaeda maritima* may be due to the presence of triterpene phyto-chemical constituents. Hence, triterpenes are proved to have hepatoprotective activity. Further studies are needed to understand the mechanism of action of extract.

### Table 1—Effect of *Suaeda maritima* on hepatoprotection on concanavalin-A induced hepatotoxicity

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose (mg/kg)</th>
<th>Hepatoprotection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Con-A treated</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Con-A+Silymarin</td>
<td>100</td>
<td>61.38</td>
</tr>
<tr>
<td>Con-A+Extract (75 mg/kg)</td>
<td>75</td>
<td>26.52</td>
</tr>
<tr>
<td>Con-A+Extract (150 mg/kg)</td>
<td>150</td>
<td>52.0</td>
</tr>
<tr>
<td>Con-A+Extract (300 mg/kg)</td>
<td>300</td>
<td>80.90</td>
</tr>
</tbody>
</table>

### Table 2—Histopathological changes in the liver of the rats treated with silymarin and plant extract

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Degeneration in hepatocytes (fatty and hydrophic changes)</th>
<th>Deformation in hepatocytes</th>
<th>Focal necrosis</th>
<th>Congestion in central vein</th>
<th>Congestion in sinusoids</th>
<th>Bleeding area in hepatic lobes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>+</td>
<td>∅</td>
<td>∅</td>
<td>+</td>
<td>∅</td>
<td>∅</td>
</tr>
<tr>
<td>Con-A treated</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Con-A+Silymarin</td>
<td>+</td>
<td>↓</td>
<td>↓</td>
<td>+</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Con-A+Extract 75 mg/kg</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Con-A+Extract 150 mg/kg</td>
<td>++</td>
<td>+</td>
<td>↓</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Con-A+Extract 300 mg/kg</td>
<td>+</td>
<td>↓</td>
<td>↓</td>
<td>++</td>
<td>+</td>
<td>↓</td>
</tr>
</tbody>
</table>

∅ absent; ↓ few; + mild; ++ moderate; +++ severe; ++++ extremely severe (the liver of 6 animals in every group were examined)
required to identify the active principles present and to establish the mechanisms of action.

Acknowledgement
Thanks are due to Indian Council of Medical Research, India for financial assistance.

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