In-vivo Hepatoprotective Activity of Methanolic Extracts of Sphaeranthus amaranthoides and Oldenlandia umbellata

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Abstract
Objective: The present study was carried out to evaluate the in vitro hepatoprotective activity of unexploited plants, Sphaeranthus amaranthoides and Oldenlandia umbellate on CCl4 induced liver injury, which are indigenous to South India. Methods: in the present study the methanolic extracts from Sphaeranthus amaranthoides and Oldenlandia umbellate were studied against the carbon tetrachloride hepatotoxicity. Results: significant hepatoprotective effect was obtained against carbon tetrachloride induced liver damage as judged from serum marker enzyme activities (SGOT, SGPT, ALT and TB) and a normal architecture of liver compare to toxic control. Conclusion: the result revealed that methanolic extracts of Sphaeranthus amaranthoides and Oldenlandia umbellate could be useful in preventing CCl4 induced liver injury.

Key words: Hepatoprotective activity, Sphaeranthus amaranthoides, Oldenlandia umbellata, CCl4, SGOT, SGPT, ALT, TB.

Introduction
Liver is the largest and most complex internal organ in the body. It plays an important role in maintenance of internal environment with the help of its multifarious and several functions. It is connected to intermediary metabolism of proteins, fats, and carbohydrates as well as in the synthesis of number plasma proteins such as albumin, fibrinogen and in the production of various enzymes, formation and excretion of bile. Drug-induced liver injury is a major health problem that remonstrance not only health care profession but also pharmaceutical industry and drug regulatory agencies. According to the United States Acute Liver Failure study group, drug-induced liver injury accounts for more than 50% of acute liver failure, including hepatotoxicity caused by overdose of acetaminophen (39%) and idiosyncratic liver injury triggered by other drugs (13%).1 Liver diseases have become one of the major causes morbidity and mortality in man and animals all over globe and hepatotoxicity due to drug appears to be the most common contributing factors.2 Among the many diseases that can affect the liver that is 'Viral Hepatitis' (inflammation of liver caused by viral infection). Hepatitis can be caused by the drugs, viruses, bacteria, mushrooms, parasite like moebias or giardiasis. In India about 40 poly herbal commercial formulations reputed to have hepatoprotective action are being used. It has been reported that 160 phyto constituents from 101 plants have hepatoprotective activity.3 Liver protective herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenoids, carotenoids, glycosides, and xanthines. Plant extracts of many crude drugs are also used for the treatment of liver disorders. Extracts of different plants of about 25 plants have been reported to cure liver disorders.4

Materials and Methods
Plant Materials and Extraction:
The plants S. amaranthoides (entire plant) and O. umbellate (entire plant) were col-
lected from the Thoothukudi district, Tamilnadu, India, voucher. No. SGIP, Ref.no: 007 and 010 in the month of December 2011 and authenticated by Dr. V. Chelladurai, retired research officer – Botany, central council for research in Ayurveda and siddha (CCRAS), government of India; Tiruneveli. Herbarium of the plants was prepared and preserved in the department of pharmacognasy, Dr. Samuel George Institute of Pharmaceutical Sciences, Markapur, Prakasam district, Andhra Pradesh. Plant materials were air dried in the laboratory for 5 days at room temperature then grinded to powder form using an electrical mill. The powder sample was kept in an air tight container until required. About 100 g of each plant sample was extracted with 500 mL methanol solvent by using soxhlet extractor. After extraction the sample was kept in dark for 72 horas with intermittent shaking. Then both the solvents were evaporated under reduced pressure using Rota-Vapor and to obtain viscous semisolid masses.

Phytochemical screening

The preliminary phytochemical screening was carried out by methanolic extract of both the plants for the presence of phyto constituents. The methanolic extract of both the plants were tested for steroids, alkaloids, phenolic compounds, flavonoids, saponins, tannins, antrhaquinone and amino acids. Phytochemical screening of both the extracts was carried out according to the standard methods.8

Acute Toxicity Studies

The acute toxicity studies for methanolic extracts of both the plants were determined on albino mice (20-30g) the plants were determined on albino rat, maintained under standard condition. The animals were fasted overnight prior to the experiment. Fixed dose method as per OECD Guideline No. 425 method, given by CPCEA was adopted for toxicity studies.7 the study was conducted by prior permission of institutional animal ethical committee (IAEC registration no. 1048/a/08/CPCSEA, approval no. 22/IAEC/SGIP/2014-15). The mice were divided in to control and test groups each containing 6 animals. The test groups of mice were administrated with the dose of 25, 200, 500, 2000 and 5000 mg/kg of extracts. Carefully observe all the mice and any sign of toxicity in the first four hours, after the administration of extracts and daily following that for the period of 14 days.

Hepatoprotective activity

Selection of animals, caring and handling

The albino rats (wistar strain 150-200 g) of either sex were used. After randomization in to various groups, animals were accustomed for a period of 10 days under standard husbandry condition. Room temperature: 23 ± 3°c Relative humidity: 50 ± 20%

12 hrs dark and light cycle.

All the animals were feed with rodent pellet diet and water was allowed ad-libitum under strict hygienic condition. Ethical clearances for performing experiments on animals are obtained from Institutional Animal Ethical Committee (IAEC registration no. 1218/a/08/CPCSEA, approval no. 17/IAEC/SGIP/2014-15). The animals were divided in to vehicle control, toxic standard and test extract of S.amaranthoides 50mg/kg, S.amaranthoides 100mg/kg, and O. umbellate 50mg/kg, O. umbellate 100mg/kg groups. Each consisting of 6 animals in all sets of experiments respectively. The drug treatment was given once daily per oral.

Study designing and experimental protocol

Albino rats of westar strain weighing 150-200 g were selected and divided in to 7 groups of each containing 6 animals. 5% gum acacia was used as a vehicle control for suspending the standard drugs and the extracts. Vehicle control (group-I) received vehicle aqueous 5% gum acacia. Group-II to Group-VII is administrated with CCL4 for 7 days at a dose of 0.25ml/100 g. Whereas the Group-II received only CCL4, Group-III receive the standard drug silymarin at a dose of 25 mg/kg b.w and Group-IV received MESA (Methanolic extract of S. amaranthoides) at a dose of 50 mg/kg b.w, Group- V received MESA (Methanolic extract of S. amaranthoides) at a dose of 100 mg/kg b.w, Group-VI received MEOU (Methanolic extract of O. umbellata) at a dose of 50 mg/kg b.w, Group-VII received MEOU (Methanolic extract of O. umbellata) at a dose of 100 mg/kg b.w. The vehicle control and all test extract administered orally for seven days at different dosage from level and enhance the liver damage in animals. On the 7th day after 6 hrs of the administration
of the drug by giving a single dose of CCl₄ with liquid paraffin (1:1 ratio). After 24 hrs blood samples were drawn from all animals by puncturing retro-orbital plexus on the day of the treatment. The blood samples were centrifuged immediately to get clear serum and subjected for estimation of various biochemical parameters namely SGPT (serum glutamic pyruvic transaminase), SGOT (serum glutamic oxaloacetic transaminase), ALP (alkaline phosphate), and TB (total bilirubin). After 24 hrs, the animals were sacrificed and liver tissues were collected from all groups for histopathological studies.

**Statistical Analysis**

Results were expressed as mean ± SEM, n=6. Statistical analysis was performed with one way analysis of variance (ANOVA) by Dunnett's multiple comparisons test. P value < 0.01 was considered to be statistically significant.

### RESULT

The present study had been attempted to demonstrate the role of hepatoprotective activity of crude methanol extracts of plant materials of *S. amaranthoids* and *O. umbellata*, belonging to the different family in CCl₄ induced hepatotoxicity at different doses. The methanolic extract of both the plants are having same phytoconstituents after prior preliminary phytochemical test but here, the difference was found to be presence of anthraquinone in *O. umbellata* when compared with *S. amaranthoids*. In the liver parameter analysis no significant changes were observed for evolution toxicity. Skin, fur, eyes, mucous membrane, behavioral pattern, salivation, sleep of the treated as well as control animals were found to be normal. Tremor, lethargy, diarrhea and coma did not occur any of the animals. There was no significant damage of the liver and no significant structural changes detected in kidney, lung, and brain from all groups of the study. Carbon tetrachloride in one of the potent hepatotoxic (toxic to the liver), and is widely used in scientific procedures. A number of reports indicate that overdose CCl₄ can produce centrilobular necrosis, focal necrosis, and congestion in central vein and congestion sinusoidal spaces were found in hepatotoxic treated rats. However *S. amaranthoids* and *O. umbellate* plant extracts (50 mg/kg b.w and 100 mg/kg b.w) pretreated rats showed reduction in fatty changes and focal necrosis, hydrophine changes. No visible changes (Figure-1) were observed.

### DISCUSSION

The present study was revealed hepatoprotective activity in rats against CCl₄ hepatotoxicity to prove its claims in folklore practice against liver disorders. A number of reports indicate that overdose CCl₄ can produce centrizonal hemorrhagic necrosis in human and experimental animals. Based on the result of acute oral toxicity studies for methanolic extracts of both the plants (S. amaranthoids and O. umbellate) would be rewarded as safe. There was no mortality found out during experimental periods of the animals. CCl₄ induced hepatic injury is commonly used as an experimental method for the study of hepatoprotective effects of medicinal plants extracts. The extent of hepatic damage is assessed by histological evolution level of various biochemical parameters in circulation. CCl₄ undergoes hepatic metabolism to give rise to tri chloro methyl radicals, which upon reacting with reactive oxygen species yields tri chloro methyl peroxide radicals. These forms covalent bond with lipids and destroy the membrane integrity. It produces hepatotoxicity by altering liver microsomal membranes in experimental animals. From the values are mean±SEM, n=6. **p<0.01, when compared with control group, *p<0.01, when compared with toxic group

### Table 1: Effect of methanolic extract of *S. amaranthoids* and *O. umbellata* on CCl₄ induced hepatotoxicity (Serum biological parameters) in albino rats.

<table>
<thead>
<tr>
<th>SL.No</th>
<th>Groups</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>Total bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (group-I)</td>
<td>119±2.82</td>
<td>101±2.20</td>
<td>187.97±1.6</td>
<td>1.58±0.14</td>
</tr>
<tr>
<td>2</td>
<td>Toxic control (group-II)</td>
<td>353.16±8.79**</td>
<td>394.82±3.14**</td>
<td>444.36±7.88**</td>
<td>4.61±0.23**</td>
</tr>
<tr>
<td>3</td>
<td>Standard group-III (25mg/kg)</td>
<td>124.86±3.39*</td>
<td>123.66±1.83*</td>
<td>184.81±2.05*</td>
<td>2.05±0.03*</td>
</tr>
<tr>
<td>4</td>
<td>M.E.S.A. group-IV (50 mg/kg)</td>
<td>352.81±2.10*</td>
<td>328.28±1.99*</td>
<td>417.94±1.97*</td>
<td>3.28±0.028*</td>
</tr>
<tr>
<td>5</td>
<td>M.E.S.A. group-V (100 mg/kg)</td>
<td>319.14±4.25*</td>
<td>338.14±2.64*</td>
<td>394.12±3.36*</td>
<td>3.07±0.18*</td>
</tr>
<tr>
<td>6</td>
<td>M.E.O.U. group-VI (50 mg/kg)</td>
<td>217.48±1.84*</td>
<td>216.45±3.98*</td>
<td>226.32±2.09*</td>
<td>2.64±0.3*</td>
</tr>
<tr>
<td>7</td>
<td>M.E.O.U. group-VII (100 mg/kg)</td>
<td>193.22±3.30*</td>
<td>211.43±2.42*</td>
<td>204.40±3.29</td>
<td>2.33±0.02*</td>
</tr>
</tbody>
</table>

Values are mean±SEM, n=6. **p<0.01, when compared with control group, *p<0.01, when compared with toxic group
significant hepatoprotection against CCL\textsubscript{4} induced hepatotoxicity in albino rats in reducing SGOT, SGPT, ALP and TB levels. Liver section of \textit{S. amaranthoides} and \textit{O. umbellate} treated animal groups clearly showed normal hepatic cells thereby confirming hepatoprotective activity.

**CONCLUSION**

In conclusion, the precious information regarding acute oral toxicity profile of both the plants would be crucial for various pharmacological screening. On the other hand methanolic extract of \textit{S. amaranthoides} and \textit{O. umbellate} could be an important source of hepatoprotective compounds specifically for CCL\textsubscript{4} induced hepatotoxicity.

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**CONFLICT OF INTEREST**

Authors declare that they have no competing interest.

**REFERENCES**
