Hepatoprotective activity of *Santolina chamaecyparissus* Linn against D-Galactosamine Induced Hepatotoxicity in Rats

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ABSTRACT: Background: *Santolina chamaecyparissus* Linn. is used traditionally in Ayurvedic system of medicine in India for the treatment of liver diseases, and as a liver tonic. Methods: The hydroalcoholic extract of *Santolina chamaecyparissus* Linn. whole plant was tested for its hepatoprotective effect against D-galactosamine induced hepatic damage in rats at a dose of 250°mg/kg. Results: The substantially elevated levels of aspartate amino transferase (ASAT), alanine amino transferase (ALAT), alkaline phosphatase (ALP), total bilirubin (TB), lactate dehydrogenase (LDH), total cholesterol (TC), triglycerides (TGL), total protein (TP) and albumin (TA) were restored significantly (p<0.001) by the extract. The hepatoprotective effect was comparable to that of standard silymarin. Results of histopathological studies supported these findings. Conclusion: It can be concluded from the present study that, the hydroalcoholic extract of *Santolina chamaecyparissus* Linn. has appreciable hepatoprotective potential. Keywords: D-galactosamine, flavanoids, hepatoprotective effect, histopathological studies, *Santolina chamaecyparissus*.

INTRODUCTION

Morbidity and mortality resulting from chronic liver diseases (such as hepatitis) is a major public health problem worldwide, especially in developing countries. The major abnormalities associated with hepatitis are lipidemia, peroxidation and loss of plasma membrane integrity. The search for new drugs to limit hepatic injury has been of recent interest. A better understanding of the processes involved in hepatitis has stimulated the clinical development of safe and non-toxic cytoprotective agents.[1] Research demonstrates that safer hepatoprotective effects are often associated with herbs.[2]

*Santolina chamaecyparissus* Linn. (family, Asteraceae) is popularly known as Lavender cotton. The plant is widely distributed throughout India on lowland plains and low hills. The whole plant is used as stimulant, vermifuge, stomachic.[3] The flowers are used for their analgesic, antispasmodic, bactericidal, fungicidal, digestive and vulnerary properties.[4] To date, hepatoprotective activity of this plant species has not been scientifically evaluated. In the present investigation, the hydroalcoholic extract of *Santolina chamaecyparissus* whole plant was screened for hepatoprotective activity in D-galactosamine induced hepatotoxicity in albino rats to evaluate the ethnomedicinal claim.

MATERIALS AND METHODS

Plant Materials

*Santolina chamaecyparissus* Linn. whole plants were collected from Mettupalayam, Coimbatore (District), Tamilnadu, India on June 2008 and authenticated by Dr. S. Rajan, Field Botanist, Central Council for Research in Homoeopathy, Ooty, Nilgiris (District), Tamilnadu, India. A voucher specimen (No.JSSCP/DPP/436) has been preserved in the Department of Phytopharmacy and Phytomedicine.
**Extraction**

The whole plant material of *Santolina chamaecyparissus* were cleaned with fresh water, dried under shade, powdered to 60 mesh size, extracted by hot decoction method with 95% v/v ethanol as the solvent. The solution was evaporated in vacuo, concentrated under reduced pressure and dried in a desiccator (Interlabs). A greenish black semi-liquid extract was formed which was dried in hot air oven to constant weight to remove any residual traces of solvent (yield 24.96% w/w).

**Phytochemical screening**

Preliminary phytochemical analysis of the extract was performed by simple chemical tests with the reagents of analytical grade.

**Estimation of total phenolic and flavonoid contents**

Total phenolic content of extract was determined using the Folin–Ciocalteau assay. Samples (300 μL) were introduced into test tubes followed by 1.5°ml of a Folin–Ciocalteau’s reagent (Qualigens) (10x dilutions) and 1.2 ml of sodium carbonate (7.5% w/v). The tubes were allowed to stand for 30 min before the absorbance was measured at 765 nm. Total phenolic content was expressed as gallic acid (Sigma) equivalent in mg/g sample.

The total flavonoid content was determined by the aluminum chloride (AlCl₃) method using rutin (Sigma) as standard. The test sample was dissolved in dimethyl sulfoxide (DMSO) (Manav Bio Chem Impex). The sample solution (150 μL) was mixed with 150 μL of 2% AlCl₃ After 10 min of incubation at ambient temperature, the absorbance of the supernatant was measured at 435 nm. Three replicates were made for each test sample. The total flavonoid content was expressed as rutin equivalents in mg/g sample.

**Pharmacological Screening**

**Acute toxicity study**

The hydroalcoholic extract was administered orally to test groups of mice at doses up to 2500 mg/kg and observed for any behavioural changes for 14 days. No lethality in was evident in any of the test groups. Hence, one tenth of the maximum dose was selected as dose for evaluation i.e. 250 mg/kg orally.

**Animals**

Healthy Wistar albino rats (180-220 g) of either sex were maintained under standard laboratory conditions and were fed with rat pellet feed ad libitum. The animal experimental protocol was approved by Institutional Animal Ethical Committee (Approval no.: JSSCP/IAEC/ M.PHARM/PHYTO/05/2008-09).

**Assessment of in vivo hepatoprotective effect**

Experimental hepatotoxicity was induced by intraperitoneal administration of D (+) galactosamine (D-Ga1N) at 400 mg/kg in 0.3% CMC (Carboxy Methyl Cellulose) in

<table>
<thead>
<tr>
<th>Groups</th>
<th>ASAT (U/L)</th>
<th>ALAT (U/L)</th>
<th>TP (g/dl)</th>
<th>LDH (U/L)</th>
<th>ALP (U/L)</th>
<th>TB (mg/dl)</th>
<th>TGL (mg/dl)</th>
<th>TC (g/dl)</th>
<th>ALB (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC</td>
<td>58 ± 3.1</td>
<td>56.0 ± 2.0</td>
<td>6.83 ± 5.8</td>
<td>14.33 ± 2.5</td>
<td>3.40 ± 0.5</td>
<td>2.45 ± 0.9</td>
<td>7.61 ± 0.5</td>
<td>57.80 ± 2.5</td>
<td>6.50 ± 1.4</td>
</tr>
<tr>
<td>D-Ga1N</td>
<td>230.1 ± 8.5</td>
<td>180.9 ± 4.3</td>
<td>2.67 ± 0.5</td>
<td>11.7 ± 11.1</td>
<td>7.32 ± 2.5</td>
<td>5.33 ± 1.5</td>
<td>3.56 ± 0.7</td>
<td>183.4 ± 4.1</td>
<td>2.54 ± 0.8</td>
</tr>
<tr>
<td>D-Ga1N + Silymarin</td>
<td>77.3 ± 1.5</td>
<td>65.0 ± 1.2</td>
<td>4.0 ± 0.2</td>
<td>6.3 ± 0.3</td>
<td>6.19 ± 0.5</td>
<td>3.1 ± 0.4</td>
<td>5.11 ± 0.1</td>
<td>76.0 ± 3.6</td>
<td>3.70 ± 0.5</td>
</tr>
<tr>
<td>D-Ga1N + SC extract</td>
<td>185.0 ± 2.6</td>
<td>185.1 ± 2.6</td>
<td>4.0 ± 0.2</td>
<td>4.0 ± 0.2</td>
<td>5.11 ± 0.7</td>
<td>3.1 ± 0.4</td>
<td>3.8 ± 0.6</td>
<td>123.8 ± 2.6</td>
<td>3.40 ± 0.5</td>
</tr>
</tbody>
</table>

Superscripts # and * indicates significant (p < 0.001) compared to solvent and negative control groups respectively. Values are expressed as mean ± SD (N = 6)
Wistar albino rats. The rats were divided into 4 groups of 6 rats each:

Group I received 0.3% CMC (10 ml/kg, orally) and served as a normal group.

Group II received 0.3% CMC (10 ml/kg, orally) and served as a control group.

Group III received Silymarin (Aurobindo/95%/w/w) (25 mg/kg, orally)

Group IV received hydroalcoholic extract of Santolina chamaecyparissus (250 mg/kg, orally).

All the animals received these treatments for a period of 14 days. On day 14 D-Ga1N (400 mg/kg, intraperitoneally) was administered to all the groups except group I animals. Twenty four hours after D-Ga1N administration, blood was collected into centrifuge tubes under light ether anaesthesia by puncturing the retro-orbital plexus. The blood was allowed to clot and separated by centrifugation at 2500 rpm. The resultant serum was analyzed for various biochemical parameters including aspartate amino transferase (ASAT, GOT), alanine amino transferase (ALAT, GPT), alkaline phosphatase (ALP), total bilirubin (TB), total protein (TP), lactate dehydrogenase (LDH), triglycerides (TGL) total cholesterol (TC) and albumin in Autoanalyzer (Microlab 200) using Ecoline diagnostic kits to determine the functional state of the liver.

Histopathological Evaluation
The liver from each animal was removed after dissection; liver lobes were fixed for 48 h in 10% formalin and were embedded in paraffin. Subsequently, 5µM sections were cut on a microtome and stained with haematoxylin and eosin were examined for histopathological changes under a light microscope and compared with normal liver histology.

Statistical Analysis
Results of estimation of biochemical and functional parameters are reported as mean ± SD. The percentage reduction in biochemical parameters was calculated by considering the differences between the hepatotoxin treated and control group as 100% level of reduction. The variation in a set of data has been estimated by performing One Way Analysis of Variance (ANOVA) and individual comparisons of group mean values were done using Dunnet’s test. The results were judged significant if P < 0.05.

RESULTS AND DISCUSSION
The total phenol and flavonoid contents of Santolina chamaecyparissus extract were found to be 13.98% and 05.60% respectively. Liver injuries can be induced by various hepatotoxins, such as D-galactosamine in rodents. This animal model is frequently used in prospective studies of hepatoprotective agents because of the experimental ease and its mechanism of action. In the hepatoprotective study, rats treated with Ga1N developed significant (p < 0.001) liver damage as observed from the elevated serum levels of hepato-specific enzymes compared to the control GaN administration induced liver injury as well as severe alteration in other biochemical parameters is indicated by increase in serum amino transferase activities and decrease in total liver proteins and increase in the amount of triacylglycerols. Treatment with hydroalcoholic extract of Santolina chamaecyparissus decreased the serum aminotransferase ASAT, ALAT and increased the total liver proteins ALP and total cholesterol in blood (Table 1). The decreased level of serum bilirubin indicates the effectiveness of the normal functional conditions of the liver. It was found that the hydroalcoholic extract offers protection against D-Ga1N induced hepatotoxicity in rats.

The histopathological studies in the transverse liver sections (Figure 1) showed normal parenchymal architecture with cords of hepatocytes, portal tracts and central veins in the control group. No cell injury or cirrhosis found. Centrilobular necrosis, accompanied by fatty changes and ballooning degeneration were seen in the remaining hepatocytes in

Figure 1: Photomicrograph of liver section (T.S.) at 100 X magnification, Haematoxylin and Eosin stain.
activities. The data obtained in the present study appears are well known for hepatoprotective and antioxidant contains phenols, flavonoids and triterpenoids. Flavonoids investigation showed that the hydroalcoholic extract to possess antihepatotoxic activity. The phytochemical thoroughly investigated of all the plant substances known of biochemical parameters and has so far been the most activity by reversing the hepatotoxin induced alterations marianum.

ACKNOWLEDGEMENTS

Authors wish to thank JSS University, Mysore and The Principal, JSS college of Pharmacy, Ooty for providing necessary facilities.

Table 2: Findings for histopathology study

<table>
<thead>
<tr>
<th>Microscopic findings</th>
<th>CMC Solvent only</th>
<th>D-GalN only</th>
<th>D-GalN + Silymarin</th>
<th>POE (250 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Architecture</td>
<td>Normal</td>
<td>Lobular disarray</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>b) Hepatocytes</td>
<td>-</td>
<td>+++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Balloon degeneration</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Foamy degeneration</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Focal necrosis</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Zone 1 necrosis</td>
<td>-</td>
<td>+ (Focal)</td>
<td>(Focal)</td>
<td></td>
</tr>
<tr>
<td>Council man bodies</td>
<td>-</td>
<td>++ (Random)</td>
<td>(Random)</td>
<td></td>
</tr>
<tr>
<td>Regeneration</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>c) Portal Tract</td>
<td>Lymphocytes</td>
<td>Lymphocytes predominant,</td>
<td>Lymphocytes</td>
<td>Lymphocytes</td>
</tr>
<tr>
<td>Nature of infiltrate</td>
<td></td>
<td>Few plasma cells, polymorphs and eosinophils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bile duct hyperplasia</td>
<td></td>
<td>+ (Focal)</td>
<td>(Focal)</td>
<td></td>
</tr>
</tbody>
</table>

+, slight; ++, marked (below 50%); ++++, very intense (overall)

REFERENCES