EVALUATION OF EFFECT OF CENTELLA ASIATICA ON CCL\textsubscript{4} INDUCED RAT LIVER DAMAGE

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Summary

Liver disorders are common but in medicinal practices reliable liver protective drugs are not available. Some herbs play important role in management of liver disorders. Centella asiatica were collected, cleaned, dried and ground to fine powder by electric mixer and sieved by BSS. The present work was carried out to investigate the hepatoprotective action of Centella asiatica plant material on CCL\textsubscript{4} (Carbon tetra Chloride) induced liver damaged in rats. Blood and tissue biochemical assays like ALT, AST, bilirubin, Total Protein, glucose etc have been studied for evaluation of hepatoprotection. From the results of these parameters it is clear that Centella asiatica gave best recovery for hepatoprotection and may be used as a potent liver tonic.

Keywords: Centella asiatica, Carbon tetra Chloride, ALT, AST, Liver Tonic.

Introduction

Liver disorder is one of the common thirst area declared by the Indian Council of Medical Research, New Delhi in the reviewed research on traditional medicine. The present work was done to evaluate the hepatoprotective effect of powder of Centella asiatica. This plant is a perennial, slender, herbaceous creeper with kidney shaped leaves of 2-5 cm diameter found in India, China and S. Africa. It is used for wound healing, bronchitis, dysentery, fever, inflammation, leucoderma and as a nerve tonic and jaundice (1).
Materials and Methods

*Centella asiatica* plants were collected from various places in Pune and Ahmednagar district; washed thoroughly and dried at room temp in shade. They were powdered, sieved through sieve of mesh to 85 (BSS) and stored in airtight containers (26, 28).

**Dose**

The dose selected for this plant powder aqueous slurry is 0.7g/Kg body weight against CCl4 damaged liver in rats, preciously for selection of amount of dose acute toxicity study has been also done in mice. All observations are found to be normal (24, 27).

**Animals**

The animals used for study were Wister Albino rats (130-150 gm) obtained from Raj Biotech (INDIA) Pvt. Ltd, Pune 411 038. They were acclimatized for 15 days before study. They were housed in polymethane cages. Each cage housed six animals and was maintained at 28 ± 2 °C.

The animals were subjected to 12 hrs cycles of light and darkness (24, 27). They were fed with commercially available feed pellets (12mm) containing crude protein (min 20-21 %), crude fiber (max 4 %), calcium (1-2 %) and phosphorus (0.6 %). Animals were supplied tap water from bottles during the experiment per day and the amount food and water intake is noted.

**Parameters Observed**

Blood of animals was collected by cardiac puncture under light ether anesthesia during sacrifice. Blood Biochemical assays were determined using a CHEMÎTO SPECTRASCAN UV 2700 spectrophotometrically. The blood parameters observed were GPT(ALT), GOT(AST), Cholesterol, Bilirubin, Triglycerides and √GT were as tissue parameters like Gycogen, T. Protein, Cholesterol, DNA, and RNA. This was done by using Standard kits supplied by Span Diagnostics Ltd., Surat, INDIA.

**Animal Grouping**

Animals were grouped into five groups. Each group with 12 animals 6 males and 6 females. Reversible liver damage was induced by 0.7 ml/Kg of CCl4 in 0.5 ml. Liquid Paraffin per animal i.p. The dose of plant powder in the form of aqueous slurry was given orally via gavages as per dose chart in table ES1(24, 27).

Gr. I served as Normal Control; Gr. II served as CCl4 Control, Gr. III served as CCl4 Recovery, Gr. IV served as CCl4 + *Centella asiatica* Plant Slurry and Gr. V served as CCl4+silymarin (a known hepatoprotectant).

The animals from all groups were sacrificed on 4th day and for of the study except the natural recovery group which was sacrificed on VIIth day after natural recovery/ regeneration of liver was initiated.
Table ES 1: Daily Doses Regime

<table>
<thead>
<tr>
<th>DAYS</th>
<th>Group I Normal control</th>
<th>Group II CCl4. control</th>
<th>Group III CCl4 treated natural recovery</th>
<th>Group IV CCl4 + plant slurry treated</th>
<th>Group V Silymarin treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5cc liq. Paraffin &amp; 2 cc d/w oral</td>
<td>0.7cc/kg CCl4 in 0.5cc liq. Paraffin i.p.&amp; 2cc d/w oral</td>
<td>0.7cc/kg CCl4 in 0.5cc liq. Paraffin i.p. &amp; 2cc d/w oral</td>
<td>0.7cc/kg CCl4 in 0.5cc liq. Paraffin i.p. &amp; 0.7 gm/kg plant slurry in 2cc d/w oral</td>
<td>0.7cc/kg CCl4 in 0.5cc liq. Paraffin i.p., 0.007gm/kg Silymarin in 2cc d/w oral</td>
</tr>
<tr>
<td>2</td>
<td>2cc d/w oral</td>
<td>2cc d/w oral</td>
<td>2cc d/w oral</td>
<td>0.7gm/kg plant slurry in 2cc d/w oral</td>
<td>0.007gm/kg Silymarin in 2cc d/w oral</td>
</tr>
<tr>
<td>3</td>
<td>2cc d/w oral</td>
<td>2cc d/w oral</td>
<td>2cc d/w oral</td>
<td>0.7gm/kg plant slurry in 2cc d/w oral</td>
<td>0.007gm/kg Silymarin in 2cc d/w oral</td>
</tr>
<tr>
<td>4</td>
<td>Sacrifice</td>
<td>Sacrifice</td>
<td>2cc d/w oral</td>
<td>Sacrifice</td>
<td>Sacrifice</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>2cc d/w oral</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>2cc d/w oral</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>Sacrifice</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

All dosages are for each individual animal in the group. The number of animals in each group 12 (6 males + 6 females). i.p.: intraperitoneal. d/w: Distilled Water.

Results and discussion

Liver damage due to CCl4

Literature survey reveals that CTC causes hepatic injury and is a well-known liver toxin. CCl4 has direct destructive effect on membranes of the hepatocyte and on consequent interface with cellular metabolism and transport. It damages the membranes of the hepatocyte causing leakage of the enzymes present in the cell. This results in elevation of the levels of plasma transaminases.

It leads to fat decomposition in the liver due to blockage of secretion of hepatic triglycerides into plasma. The toxicity of CCl4 depends upon the cleavage of C-Cl bond to generate a trichloro methyl-a free radical (CCl3O2). This cleavage occurs in the endoplasmic reticulum and is mediated by the cytochrome P-450 mixed function oxidase system. The product of the cleavage binds irreversibly to hepatic proteins and lipids. The metabolism of CCl4 releases CCl3 a free radical, which initiates per oxidation and cleavage of fatty acids in the membranes. The CCl4 derived free radicals initiates the process of peroxidations by attacking Methylene Bridge of unsaturated fatty acid side chains of microsomal lipids. This results in early morphological alteration of endoplasmic reticulum and eventually to ultimate cell death through series of changes listed below besides as yet underlined pathways like loss...
of activity of P450 xenobiotics metabolizing system, loss of glucose-6-phosphatase activity, loss of protein synthesis, loss of capacity of liver to form and excrete VLDL (Very Low Density Lipoproteins). Alterations in these parameters are used to monitor the course and extent of CCl₄ induced liver damage (8).

A single dose of CCl₄ leads to centrilobular necrosis and fatty liver. Within a few minutes, there is injury to the endoplasmic reticulum leading to functional defects of the Hepatocyte and multiple biochemical manifestations of hepatic injury. Irrespective of the route of administrations it leads to centrilobular necrosis and steatosis. Biochemical changes in the blood reflect injury. Serum enzyme levels increase with cytoplasmic enzyme reaching their peak within 12 hrs. Mitochondria enzymes reach their peak within 36 hrs. Enzymes common to both mitochondria and cytoplasm reach their peak around 24 hrs.

CTC causes accumulations of fat in the liver especially by interfering with the transfer of triglycerides from the liver into the plasma. Many clinical conditions that cause an increase in cholesterol levels also cause increase in triglycerides enzymes sensitive to cytotoxic injury are serum glycemic pyruvic transaminase (SGPT) now called Alanine amino transferase (ACT) and serum glycemic oxaloacetic transferase (SGOT) now known as Aspartate amino transferase (AST). Asparatate and Alanine amino transferases are present in high concentration in liver.

Due to hepatocyte necrosis or abdominal membrane permeability, these enzymes are released from the cells and their levels in the blood increase. ALT is a sensitive indicator to acute liver damage and elevation of this enzyme in no hepatic disease is unusual. Alkaline phosphatase, although is not a liver specific enzyme, the liver is major source of this enzyme. Also the levels of this enzyme increase in cholestasis, elevated serum gamma-glutamyl transpeptidase levels appear to be indicative of diseases of the liver, biliary tract and pancreases. Bilirubin levels in blood also increase in liver diseases. (Cirrhosis and hepatitis). Mean Tissue Biochemical Parameters for all Groups are given in table ES2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Gr.I</th>
<th>Gr.II</th>
<th>Gr.III</th>
<th>Gr.IV</th>
<th>Gr.V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gycogen</td>
<td>20.55</td>
<td>20.40</td>
<td>22.30</td>
<td>12.20</td>
<td>19.50</td>
</tr>
<tr>
<td>T. Protein</td>
<td>4.4</td>
<td>20.20</td>
<td>10.5</td>
<td>8.3</td>
<td>7.1</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1.6</td>
<td>2.30</td>
<td>1.90</td>
<td>2.10</td>
<td>1.8</td>
</tr>
<tr>
<td>DNA</td>
<td>0.5</td>
<td>0.45</td>
<td>0.90</td>
<td>1.30</td>
<td>0.6</td>
</tr>
<tr>
<td>RNA</td>
<td>2.4</td>
<td>4.90</td>
<td>3.75</td>
<td>7.00</td>
<td>6.90</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Gr.I</th>
<th>Gr.II</th>
<th>Gr.III</th>
<th>Gr.IV</th>
<th>Gr.V</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPT(ALT)</td>
<td>55.60</td>
<td>50.08</td>
<td>41.20</td>
<td>59.70</td>
<td>66.50</td>
</tr>
<tr>
<td>GOT(AST)</td>
<td>44.00</td>
<td>46.20</td>
<td>48.30</td>
<td>47.80</td>
<td>56.84</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>75.60</td>
<td>82.40</td>
<td>75.40</td>
<td>73.80</td>
<td>69.50</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>0.58</td>
<td>0.68</td>
<td>0.64</td>
<td>0.76</td>
<td>0.65</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>124.50</td>
<td>130.00</td>
<td>94.80</td>
<td>106.00</td>
<td>124.50</td>
</tr>
<tr>
<td>√GT</td>
<td>18.30</td>
<td>41.20</td>
<td>33.40</td>
<td>32.70</td>
<td>24.80</td>
</tr>
</tbody>
</table>
The results obtained from blood biochemical parameters are given in table ES3. In clinical chemistry AST, ALT, values showed significant changes. All the values were higher than those of the control animals. Similar observations were noted in bilirubin and cholesterol.

**Dosage**

1. A reversible damage was induced in rat liver by administering low concentration of CCl₄. The liver damage was induced by an intraperitonially (i.p.) injection of CCl₄ (0.7 cm³/kg body wt) liquid Paraffin to each animal of group II to V
2. An i.p injection of 0.5 cm³ of liquid Paraffin was given to each animal from Gr. I as sham treatment.
3. A dose of 0.7g/kg body wt of sieved whole plant powder suspended in 2cc/dist water was administered orally to each rat of Gr. IV
4. A dose of 0.007g/kg-body wt of silymarin (Silybon tablets manufactured by Ranbaxy lab. Ltd. India) suspended in 2CC of distilled water was administered orally to each rat of group V this dose is equivalent to the prescribed human dose of Silybon tablets.
5. The normal control group I, CCl₄ cont. Gr. II CCl₄ natural recovery group III animals were administered 2 cc D/W as show treatment except the plant powder.
6. The oral dosing was done using the gavage. The animals were first given CCl₄ injection Intraperitonially the oral dose of the drug.
7. The animals from Gr. I, II, IV and V were sacrificed at 72 hrs after CCl₄ liver administration (period of maximum liver damage)(10,13,14) and the animals from Gr. III were sacrificed on seventh day of the study.(10)

**General Observations**

Animals from all groups showed no abnormal behaviour in food and water consumptions The food consumptions of animals from CCl₄ control, CCl₄+ *Centella asiatica* plant treated and CCl₄+ silymarin group decreased significantly. The CCl₄ recovery group animals showed significant decrease up to the fourth day of the treatment, and then they showed an increase. This indicates that the animals are recovering from the toxicity induced by the CCl₄ similar observations were noted with the trends in water consumption by treated animals.

**Biochemical parameters**

CCl₄ treatment caused significant increase in plasma ALT, AST levels. There levels were not significantly recovering after natural recovery phase. The observations were competent in both the male and female animals. The plant treatment caused significant reduction in ALT and AST levels in both in male and female rats. CCl₄ treatment caused accumulation of cholesterol and the plasma levels of cholesterol were high in treated animals both in CCl₄ and CCl₄ recovery groups. *Centella asiatica* plant powder treatment significantly reduced cholesterol in all rats. Plasma levels of bilirubin significantly increased after treatment, in CCl₄ control group and CCl₄ recovery groups the levels were marginally reduced for group IV and V.

Plasma levels of triglycerides increased significantly after CCl₄ treatment. These levels remain high even after natural recovery or CCl₄ treatment but *Centella asiatica* plant slurry treatment showed significant reduction in triglycerides levels in all rats.
Plant slurry treatment caused significant reduction in cholesterol. The tissue cholesterol levels reduced after natural and Silymarin treatment. CCl₄ treatment causes classical fatty liver as indicated by significant increase in tissue cholesterol. CCl₄ treatment significantly increased plasma gamma GT levels in all treated animals. The levels decreased after plant slurry and silymarin treatment.

Total tissue protein significantly increased after CCl₄ treatment. There levels significantly decreased after natural recovery and silymarin treatment. Plant slurry treatment caused marginal reduction in total tissue proteins in rats (24, 27).

Conclusions

The present work was carried out to investigate the hepatoprotective action of *Centella asiatica* plant material on CCl₄ (Carbon tetra Chloride) induced liver damage in rats. Blood biochemical assays like GPT(ALT), GOT(AST), Cholesterol, Bilirubin, Triglycerides and γGT and tissue biochemical assays like Gycogen, T. Protein, Cholesterol, DNA, and RNA have been studied for evaluation of hepatoprotection. From the results of these parameters it is clear that *Centella asiatica* gave best recovery for hepatoprotection. The observations of “Group I” were matching with “Group IV” than all other groups. The combined synergistic effect of its constituents and micronutrients rather than to any single factor through free radicals scavenging activity play important role in regeneration of liver cells.

References