EVALUATION OF EFFECT OF *CENTELLA ASIATICA* ON CCL_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 grinded 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_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 CCl_4 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 $28 \pm 2\ ^0C$.

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 CHEMITO SPECTRASCAN UV 2700 spectrophotometrically. The blood parameters observed were **GPT(ALT), GOT(AST), Cholesterol, Bilirubin, Triglecerides 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 CCl_4 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 CCl_4 Control, Gr. III served as CCl_4 Recovery, Gr. IV served as CCl_4 + *Centella asiatica* Plant Slurry and Gr. V served as CCl_4+ silymarin (a known hepatoprotectant).

The animals from all groups were sacrificed on 4^{th} day and for of the study except the natural recovery group which was sacrificed on VII^{th} day after natural recovery/ regeneration of liver was initiated.

Table ES 1: Daily Doses Regime

DAYS	Group I Normal control	Group II CCl_4. control	Group III CCl_4 treated natural recovery	Group lV CCl_4 + plant slurry treated	GroupV Silymarin treated
1	0.5cc liq. Paraffin & 2 cc d/w oral	0.7cc/kg CCl_4 in 0.5cc liq. Paraffin i.p.& 2cc d/w oral	0.7cc/kg CCl_4 in 0.5cc liq. Paraffin i.p. & 2cc d/w oral	0.7cc/kg CCl_4 in 0.5cc liq. Paraffin i.p. & 0.7 gm/kg plant slurry in 2cc d/w oral	0.7cc/kg CCl_4 in 0.5cc liq. Paraffin i.p., 0.007gm/kg Silymarin in 2cc d/w oral
2	2cc d/w oral	2cc d/w oral	2cc d/w oral	0.7gm/kg plant slurry in 2cc d/w oral	0.007gm/kg Silymarin in 2cc d/w oral
3	2cc d/w oral	2cc d/w oral	2cc d/w oral	0.7gm/kg plant slurry in 2cc d/w oral	0.007gm/kg Silymarin in 2cc d/w oral
4	Sacrifice	Sacrifice	2cc d/w oral	Sacrifice	Sacrifice
5	-	-	2cc d/w oral	-	-
6	-	-	2cc d/w oral	-	-
7	-	-	Sacrifice	-	-

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 CCl_4

Literature survey reveals that CTC causes hepatic injury and is a well-known liver toxin. CCl_4 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 tramaminases.

It leads to fat decomposition in the liver due to blockage of secretion of hepatic triglycerides into plasma. The toxicity of CCl_4 depends upon the cleavage of C-Cl bond to generate a trichloro methyl-a free radical (CCl_3O_2). This cleavage occurs in the endoplasmic reticulum and is mediated by the cytochrome P-450 mixed function oxidase system. The product of the clevage binds irreversibly to hepatic proteins and lipids. The metabolism of CCl_4 releases CCl_3 a free radical, which initiates per oxidation and clevage of fatty acids in the membranes. The CCl_4 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-b- 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 CCl_4 induced liver damage (8).

A single dose of CCl_4 leads to centrilobular necrosis and fatty liver. Within a few minutes, there is injury to the endoplasmic reticulum lending 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 park 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 cytotomic injury are serum glytamic pyruvic transaminase (SGPT) now called Alanine amino transferase (ACT) and serum glytamic oxaloacetic transferase (SGOT) now known as Asparatate 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 phophatase, 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 ES 2: Mean Tissue Biochemical Parameters for all Groups

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

Table ES 3: Mean Blood Biochemical Parameters for all Groups

Parameter	Gr.I	Gr.II	Gr.III	Gr.IV	Gr.V
GPT(ALT)	55.60	50.08	41.20	59.70	66.50
GOT(AST)	44.00	46.20	48.30	47.80	56.84
Cholesterol	75.60	82.40	75.40	73.80	69.50
Bilirubin	0.58	0.68	0.64	0.76	0.65
Triglecerides	124.50	130.00	94.80	106.00	124.50
√GT	18.30	41.20	33.40	32.70	24.80

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 CCl_4. The liver damage was induced by an intraperitonially (i.p.) injection of CCl_4 (0.7 cm^3/kg body wt) liquid Paraffin to each animal of group II to V
2. An i.p injection of 0.5 cm^3 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, CCl_4 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 CCl_4 injection Intraperitonially the oral dose of the drug.
7. The animals from Gr. I, II, IV and V were sacrificed at 72 hrs after CCl_4 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 CCl_4 control, CCl_4+ *Centella asiatica* plant treated and CCl_4+ silymarin group decreased significantly. The CCl_4 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 CCl_4 similar observations were noted with the trends in water consumption by treated animals.

Biochemical parameters

CCl_4 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. CCl_4 treatment caused accumulation of cholesterol and the plasma levels of cholesterol were high in treated animals both in CCl_4 and CCl_4 recovery groups. *Centella asiatica* plant powder treatment significantly reduced cholesterol in all rats. Plasma levels of bilirubin significantly increased after treatment, in CCl_4 control group and CCl_4 recovery groups the levels were marginally reduced for group IV and V.

Plasma levels of triglycerides increased significantly after CCl_4 treatment. These levels remain high even after natural recovery or CCl_4 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 CCl_4 treatment causes classical fatty liver as indicated by significant increase in tissue cholesterol CCl_4 treatment significantly increased plasma gama GT levels in all treated animals. The levels decreased after plant slurry and silymarin treatment.

Total tissue protein significantly increased after CCl_4 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 CCl_4 (Carbon tetra Chloride) induced liver damage in rats. Blood biochemical assays like **GPT(ALT), GOT(AST), Cholesterol, Bilirubin, Triglecerides and √GT** 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.

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