Amelioration of CCl_4 Induced Hepatosuppression by *Tinospora cordifolia*

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Summary

The aim of the present work is to evaluate the effect of *Tinospora cordifolia* against hepatosuppression induced by carbon tetrachloride (CCl_4). The evaluation markers used were serum marker enzymes viz. GOT, GPT, Alkaline phosphate, glucose, bilirubin, Triglycerides, γ GT, cholesterol, DNA, RNA and total protein. These biochemical parameters were significantly changed by the single dose of CCl_4. The treatment of *Tinospora cordifolia* significantly recovers all the serum and liver parameters like normal levels. However, silymarin was used as a reference standard for this study. The findings indicates that the hepatoprotective action of *Tinospora cordifolia* against hepatosuppression possibly involves CCl_4 bioactivation through selective inhibitors of reactive oxygen species like antioxidants brought about significant inhibition of TBARS suggesting possible involvement of O_2^-, HO_2, HO_2^-, H_2O_2 and OH. Light and electron microscope photographs also support the same. Hence *Tinospora cordifolia* indicating protection in liver may prove promising effect against liver disorders. Thus it may act even in humans as a potent liver tonic.

Key words: *Tinospora cordifolia*, enzymes, hepatosuppression, antioxidant.

Introduction

Tinospora cordifolia (Guduchi) is a widely used shrub in folk and ayurvedic systems of medicine. The review presents a detailed survey of the literature on chemistry and medicinal properties of *Tinospora cordifolia*. The chemical constituents reported from this shrub belong to different classes such as alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds and polysaccharides[1,2,3,4]. The notable medicinal properties reported are anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immunomodulatory and anti-neoplastic activities [5, 6, 7, 8, 9].

Materials and Methods

Plant Material

Tinospora cordifolia plant material were collected around Rajgurunagar, Pune District, Maharastra, India. After collection of the required quantity, it was carefully segregated, washed and dried in shade to constant weight. The plant material was kept in preset oven for eight days at 45°C. The dried plant free of moisture was powdered in high speed electronic mixer and sieved through a BSS Mesh No. 85 sieve and stored in an airtight container. This plant material was used for animal trials.

Acute toxicity Study

The acute toxicity study of whole plant powder of *Tinospora cordifolia* was carried out on Swiss mice with a dose of 3, 5 and 7 g/Kg body weight orally. The single administration exposure of the whole plant powder in the form of aqueous slurry was carried out and the exposure route was oral with water as a vehicle. The observations of changes in body weight, food and water intake as well as cage side observations were reported. There was no mortality recorded even at the highest dose level i.e. 7g/ Kg body weight and the whole plant powder was found to be nontoxic

Animals for hepatosuppression Study

Albuno Wistar rats of either sex, weighing 130–150 g, were used. Animals were housed under controlled conditions of temperature (25 ± 2^0C) and with 12-h light/dark and fed with Amrut food pallets and tap water.

Induction of hepatic injury

Hepatic injury was induced in rats by intra-peritonial administration of a single dose of 0.7 ml/kg $CC1_4$ mixed with 0.5ml liq. Paraffin as a vehicle.

Experimental protocol

Animals were grouped into five groups and administered following dose mentioned in Table I.

Table I: DAILY DOSE REGIME

DAYS	Group I Vehicle Control	Group II CCl4 control	Group III CCl4 treated natural recovery	Group lV CCl4 + plant slurry treated	GroupV Silymarin treated
1	0.5cc liq. Paraffin & 2 cc d/w orally	0.7cc/kg CCl4 in 0.5cc liq. Paraffin i.p. And 2cc d/w orally	0.7cc/kg CCl4 in 0.5cc liq. Paraffin i.p. And 2cc d/w orally	0.7cc/kg CCl4 in 0.5cc liq. Paraffin i.p. and 0.5gm/kg plant material in 2cc d/w orally	0.7cc/kg CCl4 in 0.5cc liq. Paraffin i.p., 0.007gm/kg Silymarin in 2cc d/w orally
2	2cc d/w orally	2cc d/w orally	2cc d/w orally	0.5gm/kg plant material in 2cc d/w orally	0.007gm/kg Silymarin in 2cc d/w orally
3	2cc d/w orally	2cc d/w orally	2cc d/w orally	0.5gm/kg plant material in 2cc d/w orally	0.007gm/kg Silymarin in 2cc d/w orally
4	Sacrifice	Sacrifice	2cc d/w orally	Sacrifice	Sacrifice
5	-	-	2cc d/w orally	-	-
6	-	-	2cc d/w orally	-	-
7	-	-	Sacrifice	-	-

Note: 1. The above dosage is for an individual animal of the group.

2.The number of animals in each group = 6.

3. i.p. = intra peritoneal.

4. d/w = distilled water

5. liqd. paraffin = liquid paraffin.

Results

In present study it is observed that there was significant decrease in body weight of CCl_4 treated group as compared to normal control group given in Table II. Treatment of Silymarin and plant powder showed an increase in body weight as compared to CCl_4 treated group.

Table II: Effect o*f Tinospora cordifolia* Plant powder slurry on body weight

Groups	Body weights of rats in grams						
	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th day	7th Day
Group I Normal Control	141.4 ± 2.2	142.33 ± 3.2	144.2 ± 2.4	SACRIFICE	-	-	-
Group II CCl_4 Control	145.3 ± 3.2	144.2 ± 3.4	142.2 ± 4.0	SACRIFICE	-	-	-
Group III CCl_4 Recovery	134.4 ± 4.2	135.2 ± 3.5	137.3 ± 3.7	137.8 ± 4.0	139.1 ± 3.2	140.4 ± 2.04	SACRIFICE
Group IV Silymarin Control	143.1 ± 3.2	144.7 ± 6.20	146.7 ± 6.2	SACRIFICE	-	-	-
Group V Plant material control	149.6 ± 3.10	150.8 ± 3.2	151.9 ± 3.5	SACRIFICE	-	-	-

Blood and Tissue Biochemical Marker enzymes

All the blood biochemical marker enzymes, *viz.*, ALT, AST, Cholesterol, Bilirubin, Triglycerides, Alkaline Phosphate and GGT as well as tissue biochemical markers like glycogen, Total protein, Cholesterol, DNA and RNA reported increased activity in CCl_4 treated rats as compared to normal control group. In plant material administered group, the levels of these enzymes were found close towards normalcy. The mean values of blood and tissue biochemical parameters are given in table III.

Table III: Effect o*f Tinospora cordifolia* on Biochemical Parameters

Parameter	Gr. I Normal control	Gr. II CCl_4 control	Gr. III CCl_4 Recovery	Gr. IV Silymarin control	Gr. I Plant extract control
GPT(B)	55	63	51	70	56
GOT(B)	53	59	61	57	54
Cholesterol(B)	62	70	72	74	65
Bilirubin(B)	0.45	0.78	0.69	0.62	0.51
Triglycerides(B)	121	106	112	128	123
Gamma GT(B)	11	38	31	16	29
Alk. PO4(B)	140	163	151	148	142
Glycogen(T)	24	20	22	23	19
Total Protein(T)	05	20	10	08	07
Cholesterol(T)	02	2.2	01.8	02.1	01.7
DNA(T)	0.18	0.23	0.70	0.20	0.80
RNA(T)	2.1	4.2	3.2	4.0	2.9
Liver to Body wt Ratio	0.04	0.04	0.05	0.038	0.04

(B) : Blood Biochemical Parameter

(T) : Tissue Biochemical Parameter

The level of blood and tissue biochemical parameters reported shows significant increase in CCl_4controlled group as compared to those of normal control group. All these biochemical changes showed signs of returning more towards the normalcy in group plant material control group as compared to the natural recovery and Silymarin control group.

Discussion

Carbon tetrachloride is one of the most commonly used hepatotoxin and is well documented [13, 14, 15]. Carbon tetrachloride is biotransformed under the action of cytochrome.

The microsomal compartment of the liver to trichlomethyl radical which readily reacts with molecular oxygen to form trichloromethyloethoxy radical. This free radical in the presence of oxygen may cause peroxidation of lipid on target cell resulting in extensive damage of liver. The administration of $CC1_4$ intraperitonially to wistar rats produced hepatotoxicity showed by significant increase in the serum levels of GOT, GPT and alkaline phosphate in comparison to the control group. Also the total protein levels were significantly decreased in CCl_4 control groups from normal control group. The dose of *Tinospora cordifolia* not only prevented the rise in serum level of GOT, GPT, alkaline phosphates but also improved serum lipid profile. The results are found to be well comparable with plant material treated group[13, 14, 15] hence the plant material reports better recovery.

Liver histology

The light microscopy of normal rat liver reveals almost regular structures. The hepatocytes in thin sections appear to radiate from the central vein. The hepatocytes are polygonal with well-defined borders, with single nucleus in each. The thin sections show a portal tract with distinct endothelial lining surrounded by terminal portal venules, hepatic artery and small bile duct. (Fig. 1).

The rat liver after CCl_4 treatment shows distinct centrilobular necrosis with hepatocytes of these areas showing distinct vacuolation. The nucleus appears pynotic in these cells. The periportal region appears normal. There is distention of sinusoidal lumen in the centrilobular area. There is also distinct enlargement of hepatocytes and few areas show infiltration of mononuclear cells especially near the portal veins. (Fig. 2).

In natural recovery group the histological pictures under the light microscope revealed almost normal liver with very mild swelling of sinusoids and no congestion. The nuclei were normal indicating the recovery of the liver after the toxicant treatment. The rat liver after CCl_4 treatment in electron microscopy shows there is distinct absence of lipid accumulation and reduced mitochondrial activity as compared to CCl_4 treated cells. The microvilli appear normal. There is however, abundance of rER in hepatocytes. (Fig. 3).

The liver of the rats after combined treatment of CCl_4 and Sylimarin shows more focused regions of recovery. The dilation of sinusoids is evident in the centrilobular areas were distinctly visible. Vacuolated hepatocells and ballooned hepatocells were also seen. Congestion was significant. (Fig. 4).

The liver of the rats after combined treatment of CCl_4 and *Tinospora cordifolia* shows mild congestion in some of the sinusoids. The dilation of sinusoids is evident in the centrilobular areas. The vacuolation seen after CCl_4 treatment is significantly absent. The liver showed distinct signs of overall recovery. Bile capillaries are dialated. Focal necrotic areas were not visible with vacuolated hepatocytes. Mild congestion was seen in few areas with mononuclear cell proliferation (Fig. 5).

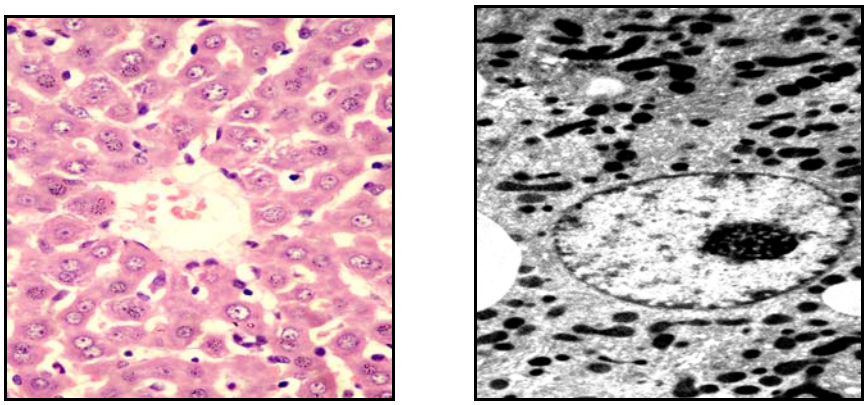

Fig. LM 1: Light micrograph and Electron Micrograph of normal rat liver

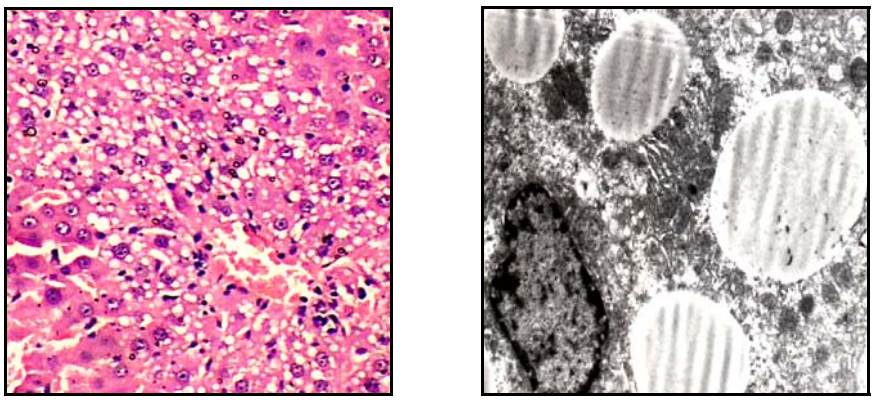

Fig. LM 2: Light micrograph and Electron Micrograph of rat liver after CCl_4 treatment

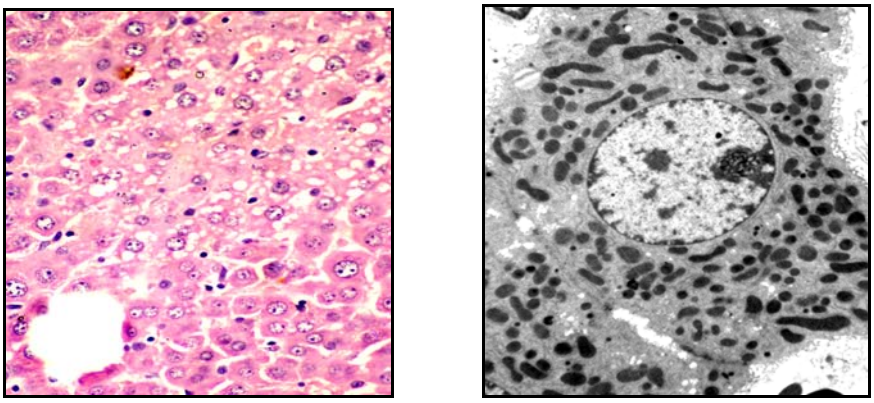

Fig. LM 3: Light micrograph and Electron Micrograph of rat liver after Natural Recovery

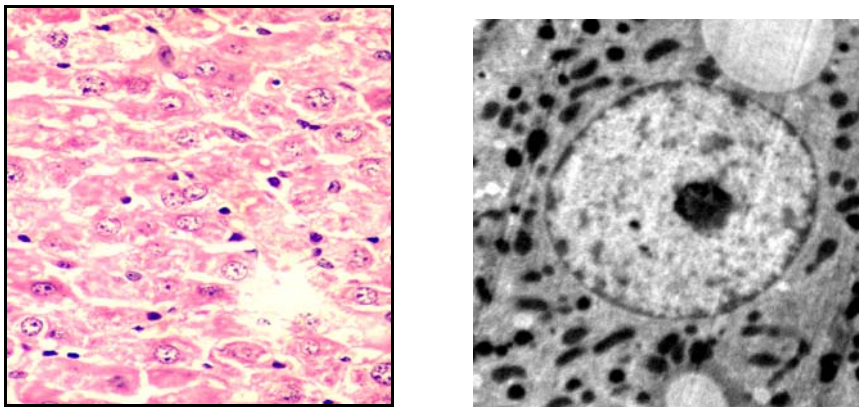

Fig. LM 4: Light micrograph and Electron Micrograph of rat liver treated with CCl_4 and Silymarin

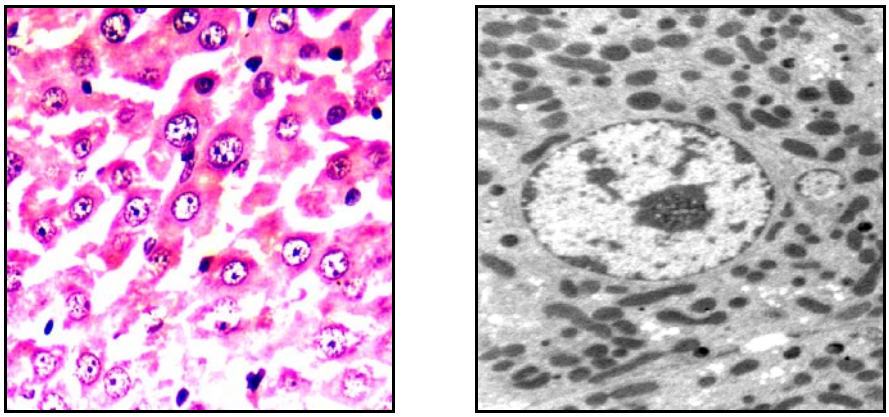

Fig. LM 5: Light micrograph and Electron Micrograph of rat liver treated with CCl_4 and plant material

Conclusions

On the basis of above findings, this may be concluded that the probable mechanism by which the *Tinospora cordifolia* plant material exerts its protective action against CCl_4-induced hepatocellular alterations through synthesis of proteins, or due to bioactivation of CCl_4 and accelerated detoxification. The potential to minimise the effects of free radicals including the peroxy radicals and its antioxidant activity in association with the inhibition of lipid peroxidation, thereby the *Tinospora cordifolia* plant material can be considered as hepatoprotective agent by the combined synergistic effect of its constituents and micronutrients rather than any single factor through free radicals activity.

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