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Protective Effect of Herbal Medicinal Plant Extract against Carbon Tetrachloride (Ccl_4)-Induced Liver Injury in Rats

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ABSTRACT

The objective of the current investigation was to investigate the hepatoprotective effect of stem of *Tinospora cordifolia* extracts against carbon tetrachloride (CCl_4)-induced liver injury in rats. The Methanolic & Hydroethanolic extracts of stem were administered orally at a dosage of 500 mg/kg body weight to Wistar albino rats, with Silymarin serving as the standard. *Methanolic and Hydroethanolic extract* shown a strong hepatoprotective effect by restoration of functional parameters, physical parameters, biochemical parameters and reducing blood enzymes alkaline phosphatase (ALP), and total bilirubin (TBL) in the chosen animal. The chemical contents of the plant include alkaloids, flavonoids, glycosides, steroids, terpenoids, phenolics, and saponins, among others. The overall experimental findings imply that bioactive phytoconstituents, such as flavonoids and alkaloids found in the *Methanolic and Hydroethanolic extracts of Tinospora cordifolia*, may be responsible for the plant's substantial hepatoprotective action. Consequently, the findings support the use of *Tinospora cordifolia* as a hepatoprotective agent.

Keywords

Carbon tetrachloride, Hepatoprotective activity, *Tinospora cordifolia*.

Introduction

The herb *Tinospora cordifolia* (Guduchi) has been utilized for centuries in traditional and alternative medical practices. It's a climber in the family Menispermaceae that grows naturally in the Indian subcontinent and China **[1]**. *Tinospora cordifolia* has long, filiform, fleshy aerial roots that grow from its branches and give the stem a succulent quality. The bark ranges in colour from ivory to grey and curls deeply to the left **[2]**. Large lenticels in the shape of rosettes may be seen dotting the smooth surfaces in between the spirals. The stem may be used to treat jaundice and other bile-related disorders since it is bitter, stomachic, diuretic **[3]**, increases bile production, induces constipation, relieves dry mouth, stomach pain, and vomiting, and prevents and treats a burning feeling in the digestive tract. Dermatological conditions may be treated with an extract from its stem **[4, 5]**. *Tinospora cordifolia* is used as an antidote for snake bites and scorpion stings when combined with other medications **[6, 7]**. *Tinospora cordifolia* root extract, when given orally to alloxan-induced diabetic rats, significantly decreased both blood glucose and brain lipids **[8]**. Reported immune system benefits of *Tinospora cordifolia* include **[9]**. In one study, goats given *Tinospora cordifolia* showed considerable improvement in clinical and hemato-biochemical markers of CCl4-

induced hepatopathy after treatment with the plant. Additionally, Hepatitis B and E surface antigens have been shown to be inactivated in vitro by *Tinospora cordifolia* extract **[10]**. Both the cotton pellet granuloma and formalin induced arthritis models benefited from the anti-inflammatory effects of *Tinospora cordifolia* aqueous extract **[11]**. Patients with rheumatoid arthritis have had their pain greatly reduced with a chemical mixture called "Rumalaya" that contains *Tinospora cordifolia*, according to a clinical review **[12]**. Diseases of the liver are a major public health concern. Herbs have a place in the treatment of liver problems since conventional medicine lacks effective medications that protect the liver. Many different plants and preparations of plants are employed in India's traditional medicine and ethnomedicine to treat liver diseases **[13]**. Due to the severe negative effects of synthetic drugs, there is a rising interest in evaluating the scientific foundation for traditional herbal treatments that are believed to contain hepatoprotective potential. Although hundreds of plants are used across the globe to prevent or treat illnesses, scientific proof in terms of contemporary medicine is missing in most situations, which is a problem for the use of medicinal plants in modern medicine. But nowadays, you need to back up your claims with scientific evidence if you want to employ a plant or its active principles **[14]**. Here, we tested the efficacy of methanol and hydroethanol extracts of *Tinospora cordifolia* on CCl4-induced liver damage in rats to see whether they might prevent or lessen the severity of the disease.

Procurement and Authentication of the Plant

The stem of *Tinospora cordifolia* were gathered from the surrounding region of Alwar, Rajasthan, and then sent to Sunrise University, Alwar, Rajasthan, where they were certified by Dept. of Botany, Sunrise University, Alwar, Rajasthan.

Preparation of extracts of *Tinospora cordifolia*

In a Soxhlet apparatus, 500g of powdered stem of *Tinospora cordifolia* were extracted with solvent in order to increasing polarity. The materials were concentrated by evaporation **[15]**.

Animals

Wistar albino rats (150-200 g) were procured from Central Drug Research Institute, Lucknow, India. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad libitum*. All the animals were acclimatized for a week before use. The experimental protocols were approved by Institutional Animal ethics Committee after scrutinization. Animals were received the drug by oral gavages tube. All the animals were care of under ethical consideration as per the CPCSEA guidelines with regular inspections of rats. The laboratory conditions duly undertaken by registered veterinary practitioner **[16]**.

Chemicals

All the chemicals and solvents were of analytical grade. Silymarin was obtained as gift sample from Micro Lbs, Goa, India. Standard kits for SGOT, SGPT and ALP etc. were obtained from Span Diagnostics Ltd., India.

Preliminary phytochemical analysis

To determine which phytoconstituents were present in each extract, a preliminary phytochemical study was performed.

Toxicity studies

All of the extracts were subjected to an acute toxicity test in accordance with OECD 423 guidelines **[16]**. Research on acute toxicity was conducted on female albino rats. Before administering the extract orally at dosages of 100, 200, and 500mg/kg b.w., the animals were fasted for 24 hours with only water provided, and then monitored for toxic symptoms for up to 72 hours. The therapeutic oral dosage for all extracts was 500 mg/kg body weight.

Carbon tetrachloride induced hepatotoxicity

The rats, of both sexes, were split up into 6 groups of six each. (n = 6) **[16, 17]**

- **Group I (Control)**: administered water (5 mililitre/kilogram, p.o.) *o.d.* for nine days.
- **Group II (-ve control):** administered water (5 mililitre/kilogram, p.o.) *o.d.* for nine days, meanwhile on the seventh day, carbon tetrachloride (1 ml/kg in 50% v/v olive oil, s.c.)

was given.

- **Group III (+ve control):** administered the normal medicine silymarin (25 mg/kg, p.o.) *o.d.* for nine days, meanwhile on the seventh day, carbon tetrachloride (1 ml/kg in 50% v/v olive oil, s.c.) was given.
- **Group IV and V (Test Sample)** administered The ***Methanolic (METC) & Hydroethanolic (HEETC)*** (500 mg/kg) extracts of stem of *Tinospora cordifolia o.d.* for nine days, meanwhile on the seventh day, carbon tetrachloride (1 ml/kg in 50% v/v olive oil, s.c.) was given.

On last day, blood was obtained from animals by puncturing retro orbital plexus. Blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and utilized for the estimation of various biochemical parameters including SGOT & SGPT **[18],** ALP **[19],** serum bilirubin **[20]** and serum protein **[21]** After collection of blood samples, the animals were sacrificed under deep ether anesthesia. Morphological parameters like weight of animals, weight of liver have also been used to evaluate the protective effect of the drug. Hepatoprotective chemical causes loss in liver weight/100 gm body weight of rats **[22, 23]**.

Histopathology studies

A portion of liver tissue of all the animal groups was excised and then washed with normal saline. The liver tissues were fixed in 10% buffered neutral formalin for 48 hrs and then with bovine solution for 6 hrs and were then processed for paraffin embedding. By using a microtome, sections of 5 mm thickness were taken and stained with hematoxylin and eosin. These sections were examined under light microscope using a magnification of 100X **[24]**.

Statistical Significance

The results of the study were expressed as mean ± SEM, n=6. ANOVA **[25]** was used to analyze and compare the data, followed by Dunnet's **[26]** test for multiple comparisons.

Results

Chemical testing identified the presence of the phytoconstituents in the different extracts. The findings demonstrate that ***Methanolic (METC) & Hydroethanolic (HEETC)*** extract of stem of *Tinospora cordifolia* contain the greatest amount of pharmacologically active compounds, such as glycosides, sponins, phytosterols, and flavonoids. As a result, these extracts were chosen for the pharmacological research. The findings are shown in **Table 1.** There was no mortality found amongst the graded dose groups of animals and they did not show any toxicity or behavioural changes at a dose level of 5000 mg/kg. This finding suggests that ***Methanolic (METC) & Hydroethanolic (HEETC)*** (500 mg/kg) extracts of stem of *Tinospora cordifolia* were safe or non-toxic to rats and hence doses of 500 mg/kg, *p.o.* were selected for the study. All groups of animals tested fell asleep after receiving an intramuscular injection of thiopentone sodium (40 mg/kg). When CCl_4 was administered to rats, the beginning of sleep was significantly delayed (measured in seconds) and the total amount of time spent in sleeping was increased (measured in minutes). Pretreatment with ***Methanolic (METC) & Hydroethanolic (HEETC)*** extract of *Tinospora Cordifolia* Stem (500 mg/kg, p.o.) and silymarin, substantially improved sleep onset but dramatically reduced sleep duration in rats compared to a CCl_4 treatment group. The outcomes are shown in **Table 2.** An increase in liver weight and liver volume were seen in the CCl_4 treated group, indicating that the livers of these individuals had grown in size. Liver weight was significantly restored in the groups given ***Methanolic (METC) & Hydroethanolic (HEETC)*** extract of *Tinospora Cordifolia* Stem (500 mg/kg, p.o.) together with silymarin. The findings are shown in **Table 3.** There was a significant elevation in the levels of serum marker enzymes like SGOT, SGPT and ALP content of CCl_4 treated groups, But pretreatment with ***Methanolic (METC) & Hydroethanolic (HEETC)*** extract of *Tinospora Cordifolia* Stem (500 mg/kg, *p.o.*) and silymarin (25 mg/kg, *p.o.*) exhibited an ability to counteract the hepatotoxicity by decreasing serum marker enzymes. The findings are shown in **Table 4.** In CCl_4 treated groups, there was a significant increase in total bilurubin and significant reduction in total protein content.

Whereas, pretreatment with ***Methanolic (METC) & Hydroethanolic (HEETC)*** extract of *Tinospora Cordifolia* Stem (500 mg/kg, p.o.) caused significant reduction in total bilirubin and significant increase in total protein. The findings are shown in **Table 4**.

Histopathological studies of liver also provided a supportive evidence for biochemical analysis. Histological changes such as steatosis (fatty changes in hepatocytes) and perivenular fibrosis were observed in CCl_4 treated (toxic) control group. Both the extracts has prevented these histological changes. The results were showed in **Figure 1**.

Table 1: Preliminary Phytochemical studies of Extracts of *Tinospora Cordifolia* Stem

Constituents	*Methanolic Extract (METC)*	*Hydroethanolic Extract (METC)*
Carbohydrate	+	+
Glycosides	-	-
Oil and fats	-	+
Proteins	+	+
Saponins	-	-
Phenolic comp. and tannins	+	+
Phytosterols	+	+
Alkaloids	+	+
Gums and mucilage	+	-
Flavonoids	+	+

Table 2: Effect of *Methanolic (METC) & hydroethanolic (HEETC)* extract of *Tinospora cordifolia* stem on functional parameters in CCl_4 induced hepatotoxic rats.

Treatment/ Dose	Onset of sleep(Sec.)	Duration of sleep (Min.)
Normal	168.7 ± 3.10	105.4 ± 3.52
Induced (CCl_4)	73.12 ± 3.47*	247.5 ± 9.12*
Standard (Silymarin)	156.7 ± 3.12***	147.2 ± 2.17***
METC (500 mg/kg)	105.2 ± 3.30**	185.12 ± 7.40**
HEETC (500 mg/kg)	145.01 ± 5.67***	165.8 ± 4.70***

Values are mean ± SEM, n = 6. (One way ANOVA Followed by Dunnette multiple comparisons test). Statistically significance of ** P<0.01, *** P<0.001, when compared with CCl_4 induced group and * P<0.05, when compared with normal group.

Table 3: Effect of *Methanolic (METC) & hydroethanolic (HEETC)* extract of *Tinospora cordifolia* stem on Physical Parameters in CCl_4 induced hepatotoxic rats.

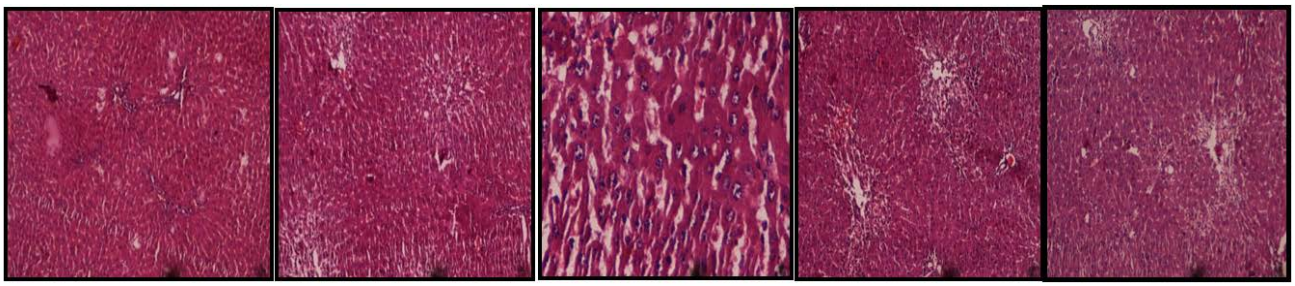
Treatment/ Dose	Liver Weight	Liver Volume
Normal	6.45 ± 0.07	6.49 ± 0.08
Induced (CCl_4)	10.02 ± 1.26*	10.12 ± 2.21*
Standard (Silymarin)	6.97 ± 1.10***	7.14 ± 1.70***
METC (500 mg/kg)	8.20 ± 1.30**	8.35 ± 1.48**
HEETC (500 mg/kg)	7.77 ± 1.38***	7.92 ± 1.60***

Values are mean ± SEM, n = 6. (One way ANOVA Followed by Dunnette multiple comparisons test). Statistically significance of ** P<0.01, *** P<0.001, when compared with CCl_4 induced group and * P<0.05, when compared with normal group.

Table 4: Effect of *Methanolic (METC) & hydroethanolic (HEETC)* extract of *Tinospora cordifolia* stem on serum enzyme parameter in CCl_4 induced hepatotoxic rats.

Treatment/ Dose	SGPT U/L	SGOT U/L	ALP U/L	Total Bilirubin (mg/dl)	Total Protein (gm/dl)
Normal	63.12 ± 3.71	167.25 ± 2.70	184.21 ± 8.02	0.43 ± 0.07	9.84 ± 0.23
Induced (CCl_4)	189.21 ± 8.23*	397.21 ± 8.24*	387.15 ± 7.55*	6.72 ± 7.04*	4.85 ± 0.16*
Standard (Silymarin)	70.12 ± 3.41***	175.84 ± 4.57***	192.14 ±8.26***	0.48 ±2.67***	9.64 ± 4.70***
METC (500 mg/kg)	91.25 ± 3.65***	258.18 ± 4.63***	230.14± 6.31***	0.64 ± 4.33***	7.91 ± 4.05**
HEETC (500 mg/kg)	81.24 ± 5.58***	191.25 ± 8.54***	214.52 ± 8.27***	0.52 ± 0.07	8.99 ± 0.23

Values are mean ± SEM, n = 6. (One way ANOVA Followed by Dunnette multiple comparisons test). Statistically significance of ** P<0.01, *** P<0.001, when compared with CCl_4 induced group and * P<0.05, when compared with normal group.



NORMAL CONTRO **SILYMARIN (25 mg/kg)** **CCL_4 INDUCED** ***METC* (500 mg/kg)** ***HEETC* (500mg/kg)**

Discussion

There are many factors which are responsible for the liver damage or injuries such as chemicals and drugs. In the present study, CCl_4 was used to induce hepatotoxicity, since it is clinically relevant. Elevated levels of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) are indications of hepatocellular injury **[27]**.

In CCl_4 induced hepatotoxicity, CCl_4 is metabolized in human cell (endoplasmic reticulum and mitochondria) with the formation of CCl3O-, the reactive oxidative free radical intermediate generated by cytochrome P450. The nascent oxygen O- resulting from lipoperoxidation causes an increase in intracellular reactive Fe+2 ions, aldehyde, GSH depletion, and calcium restoration. In addition to direct covalent contact, oxidative CCl3 O- causes degeneration of Ca+2 sequestrations. Failure to sequester leads in increased intercellular Ca+2, aggregation by proteolytic enzymes, and a rise in Fe+2 ions, which precipitates aldehyde cytotoxicity through lipid peroxidation **[28]**.

Administration of CCL_4 to experimental animals produced statistically significant rise in the enzymes levels, namely SGOT, SGPT, ACP, ALP, etc indicating the chemical induced hepatocellular toxicity. The inhibitory effect of the ***Methanolic (METC) & Hydroethanolic (HEETC)*** extract of *Tinospora Cordifolia* Stem (500 mg/kg, p.o.) on hepatotoxicity were compared to that of positive control group. The significant protection in the biochemical parameters like SGOT, SGPT, ACP and ALP against CCl_4 induced elevations in pretreatment of the animals with the ***Methanolic (METC) & Hydroethanolic (HEETC)*** extract of *Tinospora Cordifolia* Stem (500 mg/kg, p.o.). Further there was increase in weight of the liver treated with the CCl_4 were seen as compared to the normal. The treatment with the ***Methanolic (METC) & Hydroethanolic (HEETC)*** extract of *Tinospora Cordifolia* Stem (500 mg/kg, p.o.) retains the liver weight near to the normal. Liver section of control rat showing a normal hepatic architecture wall brought about from the central vein. The liver samples of CCl_4 treated rats showed gross necrosis of the centrilobular hepatocytes characterized by gross necrosis, degeneration, karyolysis and eosinophilic infiltration which are significantly prevented by treatment with the ***Methanolic (METC) &***

Hydroethanolic (HEETC) extract of *Tinospora Cordifolia* Stem (500 mg/kg, p.o.) that showed the hepatoprotective activity. The histopathological pattern of the livers of the rats treated with CCl_4 plus extracts showed minimal necrosis in centrilobular and regeneration of hepatocytes. A number of scientific reports indicated that certain flavonoids, triterpenoids and steroids have protective effect on liver due to its antioxidant properties. Administration of ***Methanolic (METC) & Hydroethanolic (HEETC)*** extract of *Tinospora Cordifolia* Stem (500 mg/kg, p.o.) that showed significant hepatoprotective activity; while qualitative phytochemical investigations on the ***Methanolic (METC) & Hydroethanolic (HEETC)*** extract of *Tinospora Cordifolia* Stem (500 mg/kg, p.o.) also showed test positive for flavonoids by chemical tests. Further, it has been reported that the flavonoid constituents of the plant possess antioxidant properties and was found to be useful in the treatment of liver damage .**[29]**

The administration of hepatoprotective drugs may induce the hepatocytes to resist the toxic effect of CCl_4. The results indicated that the ***Methanolic (METC) & Hydroethanolic (HEETC)*** extract of *Tinospora Cordifolia* Stem (500 mg/kg, p.o.) has significant hepatoprotective activity. The obtained results indicated a high degree of protection against the hepatotoxic effect of CCl_4.

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