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Research Article

Hepatoprotective effect of Herbal Medicinal Plant Extract against Paracetamol Induced Liver Injury in Rats

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Abstract:

The purpose of this study was to examine the hepatoprotective efficacy of *Tinospora Cordifolia* stem extracts against Paracetamol-induced liver damage in rats. Wistar albino rats were orally fed 500 mg/kg of body weight of Methanolic and Hydroethanolic stem extracts, with Silymarin serving as the reference. By restoring functional parameters, physical parameters, biochemical parameters, and decreasing blood enzymes alkaline phosphatase (ALP) and total bilirubin (TBL) in the selected animal, the methanolic and hydroethanolic extracts exhibited a potent hepatoprotective effect. The plant's chemical composition includes, among others, alkaloids, flavonoids, glycosides, steroids, terpenoids, phenolics, and saponins. The overall experimental data suggest that bioactive phytoconstituents, such as flavonoids and alkaloids, present in the Methanolic and Hydroethanolic extracts of *Tinospora Cordifolia* may be responsible for the plant's significant hepatoprotective effect. The results thus support the use of *Tinospora Cordifolia* as a hepatoprotectant.

Keywords

Carbon tetrachloride, Hepatoprotective activity, *Tinospora Cordifolia*.

Introduction

Tinospora Cordifolia (Guduchi) has been employed in traditional and alternative traditional medicinal for ages. It is a Menispermaceae family climber that is native to the Indian subcontinent and China [1]. The stem of *Tinospora Cordifolia* is succulent due to its long, filiform, fleshy aerial roots that develop from its branches. The bark's hue varies from white to grey, and it curves strongly to the left [2]. Large lenticels resembling rosettes are seen on the flat surfaces between the spirals. Since it is

bitter, stomachic, diuretic [3], promotes bile production, produces constipation, soothes dry mouth, stomach discomfort, and vomiting, and avoids and remedies a burning sensation in the digestive system, the stem may be used to treat jaundice and other bile-related ailments. Dermatological disorders may be treated with a stem extract [4, 5]. When combination with other treatments, *Tinospora Cordifolia* is useful as an antidote for snake bites and scorpion stings [6, 7]. Oral administration of *Tinospora Cordifolia* root extract to alloxan-induced diabetic mice dramatically lowered blood glucose and brain lipids [8]. *Tinospora Cordifolia* has been credited with immune system advantages [9]. In one research, goats treated with *Tinospora Cordifolia* shown significant improvement in clinical and hemato-biochemical indicators of **Paracetamol**-induced hepatopathy. Additionally, *Tinospora Cordifolia* extract has been demonstrated to inhibit Hepatitis B and E surface antigens in vitro [10]. The anti-inflammatory benefits of *Tinospora Cordifolia* aqueous extract [11] were shown in both cotton pellet granuloma and formalin-induced arthritic models. According to a clinical evaluation [12], patients with rheumatoid arthritis who took the chemical combination "Rumalaya" including *Tinospora Cordifolia* saw a significant reduction in discomfort. Liver disorders are an important public health problem. Herbs have a role in the treatment of liver disorders since contemporary medicine lacks effective liver-protecting drugs. In India's traditional medicine and ethnomedicine, several herbs and plant compounds are used to cure liver problems [13]. There is a growing interest in studying the scientific basis for traditional herbal therapies that are considered to have hepatoprotective potential due to the significant adverse effects of synthetic medications. Although hundreds of plants are used to prevent or cure sickness on a global scale, scientific evidence in the context of current medicine is lacking in the majority of cases, which poses a dilemma for the use of medicinal plants in contemporary medicine. To use a plant or its active components nowadays, however, you must provide scientific proof to support your claims [14]. Here, we evaluated the ability of methanol and hydroethanol *Tinospora Cordifolia* extracts to prevent or minimize the severity of Paracetamol induced liver damage in rats.

Procurement and Authentication of the Plant

The stems of *Tinospora Cordifolia* were collected from the area around Alwar, Rajasthan, and then submitted to the Department of Botany at Sunrise University, Alwar, Rajasthan, where they were authenticated.

Preparation of extracts of *Tinospora Cordifolia*

In order to increase polarity, 500g of powdered *Tinospora Cordifolia* stem were extracted using a Soxhlet device and a solvent. By means of evaporation, the materials were concentrated [15].

Animals

The Central Drug Research Institute in Lucknow, India, supplied 150-200 g Wistar albino rats. The animals were given a normal pellet diet (Hindustan lever Ltd., Bangalore) and had access to drink at will. Each animal was acclimated for one week prior to usage. After examination, the Institutional Animal Ethics Committee authorized the experimental procedures. The medication was administered to the animals using an oral gavage tube. All animals were cared for ethically in accordance with CPCSEA norms, and rats were subjected to frequent inspections. The laboratory settings are supervised by a licensed veterinarian [16].

Chemicals

All chemical and solvent was of analytical quality. Micro Lbs in Goa, India, provided a complimentary sample of silymarin. India's Span Diagnostics Ltd. supplied standard kits for SGOT, SGPT, ALP, and others.

Preliminary phytochemical analysis

An initial phytochemical analysis was conducted to establish which phytoconstituents were present in each extract.

Toxicity studies

According to the OECD 423 guidelines [16], all of the extracts were submitted to an acute toxicity test. Albino female rats were used for research into acute toxicity. Before giving the extract orally at doses of 100, 200, and 500mg/kg bw, the animals were fasted for 24 hours with just water and then observed for up to 72 hours for toxic signs. All extracts had a therapeutic oral dose of 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, paracetamol (1 g/kg p.o.) was administered.
- **Group III (+ve control):** administered the normal medicine silymarin (25 mg/kg, p.o.) *o.d.* for nine days, meanwhile on the seventh day, paracetamol (1 g/kg p.o.) was administered.
- **Group IV and V (Test Sample)** administered The M**ethanolic (METC) & Hydroethanolic (HEETC)** (500 mg/kg) extract of stem of *Tinospora Cordifolia o.d.* for nine days, meanwhile on the seventh day, paracetamol (1 g/kg p.o.) was administered.

Blood was collected from animals on the last day by puncturing the retro orbital plexus. Blood samples were allowed to clot at room temperature for 45 minutes. SGOT & SGPT [18], ALP [19], serum bilirubin [20], and serum protein [21] were measured after serum was separated by centrifugation at 2500 rpm at 30°C for 15 minutes and used for the assessment of different biochemical parameters. After the collection of blood samples, the animals were sacrificed while deeply sedated with ether. In evaluating the drug's preventive impact, morphological data such as animal weight and liver weight have also been considered. The hepatoprotective chemical decreases the liver weight per 100 g of rat body weight [22, 23].

Histopathology studies

In each group of animals, a small piece of liver tissue was removed and washed in normal saline. The liver tissues were paraffin embedded after being fixed in 10% buffered neutral formalin for 48 hours and then in bovine solution for 6 hours. Staining with hematoxylin and eosin was performed on slices that were 5 mm thick and cut using a microtome. These slices were looked at using a light microscope at 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

The phytoconstituents in the various extracts were detected using chemical analysis. Results show that the highest concentrations of pharmacologically active chemicals, including glycosides, sponins, phytosterols, and flavonoids, are found in the Methanolic (METC) and Hydroethanolic (HEETC) extract of *Tinospora Cordifolia*'s stem. In

light of this, the pharmacological studies choose to focus on these extracts. Table 1 displays the results. At a dosage of 5000 mg/kg, there was no toxicity or behavioural alterations seen in the animal groups that were subjected to the graduated dose. This result indicates that 500 mg/kg, p.o. of Methanolic (METC) & Hydroethanolic (HEETC) extracts of stem of *Tinospora Cordifolia* were safe or non-toxic to rats. After receiving a thiopentone sodium (40 mg/kg) intramuscular injection, all test groups exhibited sleepiness. Rats given **Paracetamol** slept for longer periods overall and had considerably later sleep onset times (measured in minutes). *Tinospora Cordifolia* stem methanolic (METC) and hydroethanolic (HEETC) extract (500 mg/kg, p.o.) and silymarin significantly enhanced sleep start but significantly decreased sleep duration in rats compared to a **Paracetamol** treatment group. The results are shown in Table 2. Both the weight and volume of the livers of those who were given **Paracetamol** showed growth, suggesting that the livers of those people had enlarged. *Tinospora Cordifolia* stem extract (METC) and hydroethanolic extract (HEETC) (500 mg/kg, p.o.) substantially recovered liver weight compared to the control group. The results are shown in Table 3. However, pretreatment with ***Methanolic (METC) & Hydroethanolic (HEETC) extract of Tinospora Cordifolia*** Stem (500 mg/kg, p.o.) and silymarin (25 mg/kg, p.o.) showed a capacity to prevent the hepatotoxicity by reducing serum marker enzymes. Findings are shown in Table 4. An elevation in total bilurubin and a decrease in total protein content were observed in **Paracetamol**-treated groups. Total bilirubin was significantly decreased and total protein was significantly increased after pretreatment with a Methanolic (METC) and Hydroethanolic (HEETC) extract of *Tinospora Cordifolia* stem (500 mg/kg, p.o. Outcomes are shown in Table 4.Evidence for biochemical analysis was also revealed by histopathological investigations of liver tissue. Paracetamol treated (toxic) controls showed histological alterations including steatosis (fatty changes in hepatocytes) and perivenular fibrosis. These histological alterations were stopped by both extracts. Figure 1 displays the obtained data.

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 Paracetamol induced hepatotoxic rats.

Treatment/ Dose	Onset of sleep(Sec.)	Duration of sleep (Min.)
Normal	171.2 ± 2.10	107.01 ± 1.60
Paracetamol	85.4 ± 6.26*	254.7 ± 5.92*
Silymarin	142.8 ± 4.50***	128.8 ± 4.50***
METC (500 mg/kg)	129.05 ± 4.85**	225.0 ± 5.10**
HEETC (500 mg/kg)	137.4 ± 5.74***	185.82 ± 5.80***

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 **Paracetamol** 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 Paracetamol induced hepatotoxic rats.

Treatment/ Dose	Liver Weight	Liver Volume
Normal	6.81 ± 0.07	6.95 ± 0.05
Paracetamol	9.48 ± 0.66*	9.42 ± 0.49*
Silymarin	7.10 ± 0.45***	7.13 ± 0.49***
METC (500 mg/kg)	8.16 ± 0.24***	8.24 ± 1.28**
HEETC (500 mg/kg)	7.91 ±0.80***	7.85 ± 0.90***

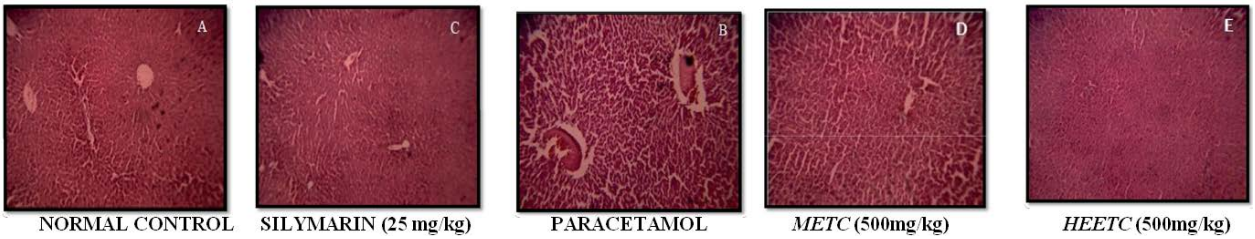
Values are mean ± SEM, n = 6. (One way ANOVA Followed by Dunnette multiple comparisons test). Statistically significance of ** P<0.01, *** P<0.001, when compared with **Paracetamol** induced group and * P<0.05, when compared with normal group.

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

Treatment/ Dose	SGPT U/L	SGOT U/L	ALP U/L	Total Bilirubin (mg/dl)	Total Protein (gm/dl)
Normal	65.1 ± 3.74	165.05 ± 2.70	182.0 ± 8.02	0.49 ± 0.07	9.75 ± 0.23
Paracetamol	191.31 ± 7.43*	368.69 ± 8.44*	352.43 ± 7.55*	6.68 ± 7.04*	5.59 ± 0.16*
Silymarin	76.50 ± 3.41***	174.71 ±4.56***	205.27 ±8.36***	0.52 ± 2.57***	9.65 ± 4.70***
METC(500mg/kg)	141.02 ± 3.65***	262.01 ± 4.63***	234.14 ± 6.41***	0.67 ± 4.33***	7.85 ± 4.05**
HEETC(500mg/kg)	141.21 ± 4.76***	195.0 ± 9.46***	202.22 ± 8.66***	0.62 ± 0.58***	8.18 ± 1.48***

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 **Paracetamol** induced group and * P<0.05, when compared with normal group.

Figure 6.14: Effect Of *Methanolic (Metc) & Hydroethanolic (Heetc) Extract Of Tinospora Cordifolia* Stem On Histopathological Diagram Of Liver Tissue In Paracetamol Induced Hepatotoxic Rats.



Discussion

Many variables, such as chemicals and medicines, may cause liver damage or injury. Because it is clinically relevant, **Paracetamol** was employed to produce hepatotoxicity in this investigation. Elevated levels of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) are indicators of hepatocellular damage [27].

Paracetamol is a common antipyretic and analgesic. Several research in animals and people have shown that greater dosages of acetaminophen cause liver damage. Paracetamol-induced hepatotoxicity has been utilized as a reliable approach for screening Hepatoprotective drugs. Paracetamol is largely metabolized in the liver and excreted by the kidney after conjugation with sulphate and glucuronide. Furthermore, paracetamol hepatotoxicity has been linked to the creation of hazardous metabolites when a portion of the drug is converted by hepatic cytochrome P-450 to the highly reactive metabolite N acetyl-p-benzoquinoneimine (NAPQI). Toxic metabolites (N-acetyl-p-benzoquineimine) have the ability to alkylate and oxidize intracellular GSH, resulting in liver damage. GSH depletion causes enhanced lipid peroxidation by extracting hydrogen from a polyunsaturated fatty acid and, eventually, liver injury owing to larger paracetamol dosages. Reactive metabolites may cause early cell stress by a variety of methods, including glutathione (GSH) depletion or binding to enzymes, lipids, nucleic acids, and other cell components. AST and ALP are normally seen in significant concentrations in the liver. These enzymes are released from the cells as a result of hepatocyte necrosis or aberrant membrane permeability, and their levels in the blood rise. ALT is a sensitive marker of acute liver injury, and its rise in non-hepatic illnesses is rare. ALT is a more specific hepatic paranchymal enzyme than AST. **[28]**.

The administration of paracetamol to experimental animals resulted in a statistically significant increase in the levels of enzymes such as SGOT, SGPT, ACP, ALP, and others, suggesting chemical-induced liver injury. The inhibitory impact of *Tinospora Cordifolia* Stem Methanolic (METC) and Hydroethanolic (HEETC) extracts (500 mg/kg, p.o.) on hepatotoxicity was compared to that of the positive control group. Pretreatment of the animals with ***Methanolic (METC) & Hydroethanolic (HEETC) extract of Tinospora Cordifolia*** Stem (500 mg/kg, p.o.) provided considerable protection in biochemical parameters such as SGOT, SGPT, ACP, and ALP against **Paracetamol**-induced increases. Furthermore, there was an increase in the weight of the liver treated with **Paracetamol** when compared to the control. The ***Methanolic (METC) & Hydroethanolic (HEETC) extract of Tinospora Cordifolia*** Stem (500 mg/kg, p.o.) therapy keeps the liver weight close to normal. A control rat's liver segment reveals a normal hepatic architectural wall caused by the central vein. The liver samples of **Paracetamol**-treated rats showed gross necrosis of the centrilobular hepatocytes characterized by gross necrosis, degeneration, karyolysis, and eosinophilic infiltration, which was significantly prevented by treatment with the ***Methanolic (METC) & Hydroethanolic (HEETC) extract of Tinospora Cordifolia*** Stem (500 mg/kg, p.o.) that demonstrated hepatoprotective activity A number of scientific studies have shown that some flavonoids, triterpenoids, and steroids have antioxidant characteristics that protect the liver. Administration of ***Methanolic (METC) & Hydroethanolic (HEETC) extract of Tinospora Cordifolia*** Stem (500 mg/kg, p.o.) that demonstrated significant

hepatoprotective activity; while qualitative phytochemical investigations on the ***Methanolic (METC) & Hydroethanolic (HEETC) extract of Tinospora Cordifolia*** Stem (500 mg/kg, p.o.) that demonstrated positive flavonoids by chemical tests. Furthermore, it has been observed that the plant's flavonoid contents have antioxidant qualities and have been proven to be effective in the treatment of liver damage. [**29]**

Treatment with hepatoprotective medications may help the liver's hepatocytes endure **Paracetamol**'s damaging effects. The findings showed that *Tinospora Cordifolia* stem extract (500 mg/kg, p.o.) exhibits considerable hepatoprotective action when tested in methanolic (METC) and hydroethanolic (HEETC) solvents. The data showed that the hepatotoxic effects of Paracetamol were significantly reduced.

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