International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 11, Issue 5, pp: 68-81, 2026
https://doi.org/10.55126/ijzab.2026.v11.i05.010 **http://www.ijzab.com**

Research Article

HPTLC FINGERPRINTING AND STANDARDIZATION OF *TINOSPORA CORDIFOLIA* STEM FOR HEPATOPROTECTIVE ACTIVITY

[1]K.V. Parthasarathi, [2]Syed Siraj Alam Hussaini, [3*] Tabrej Mujawar, [4]Mohd Wasiullah, [5]Akash kumar, [6]Deeksha Rai, [7]Yashika Bidhuri, [8]Ankita Singh

[1]Department of Pharmaceutical Chemistry, KS Rangasamy College of Pharmacy, The Tamil Nadu Dr. MGR medical University Chennai, Tamil Nadu, India
[2]Department of Pharmacology, Prathap Narender Reddy College of Pharmacy Peddashapur, Shamshabad, Hyderabad Pin code:509325.
[3]Department of Pharmacology, Gangamai College of Pharmacy, Nagaon Dhule MS India Pin- 424005.
[4]Prasad Institute of Technology, Department of Pharmacy, Punch Hattia Sadr, Jaunpur, U.P. - 222001, India.
[5]Department of Ayurved Samhita Evum Siddhanta, Govt. Ayurvedic College and Hospital Patna, Bihar 800003.
[6]Department of Pharmacognosy, Yenepoya Pharmacy College & Research centre, Yenepoya (Deemed to be University), Mangalore, Karnataka, 575017.
[7]Department of Dravyaguna Vigyan, Ishan Ayurvedic Medical College Greater Noida Uttar Pradesh 201310.
[8]Department of Pharmacognosy, Bihar College of Pharmacy, Patna, Bihar, 801503.

Article History: Received 29th May 2026; Accepted 24th July 2026; Published 1st September 2026

ABSTRACT

High-performance thin layer chromatography combined with in vivo evaluation offers a robust framework for validating herbal hepatoprotectives such as *Tinospora cordifolia*. In the present work, an ethanolic stem extract was standardized through phytochemical screening and High-performance thin layer chromatography fingerprinting, then assessed against paracetamol-induced liver injury in Wistar rats. Authenticated plant material was extracted, chemically profiled, and administered orally at 200 and 400 mg/kg for seven days. Biochemical markers and histopathology were evaluated, with silymarin serving as reference. The extract revealed abundant alkaloids, diterpenoid lactones, flavonoids, and phenolics, while High-performance thin layer chromatography produced reproducible bands corresponding to key marker compounds, confirming quality and identity. Paracetamol markedly elevated liver enzymes and disrupted hepatic architecture; treatment with *T. cordifolia* significantly restored biochemical balance and preserved tissue structure in a dose-dependent manner, approaching silymarin at the higher dose. Protective effects appear related to antioxidant reinforcement, membrane stabilization, and modulation of inflammatory responses. The integrative approach links chemical fingerprints with biological performance, strengthening the scientific basis for standardization, authentication, and therapeutic consistency of herbal preparations. Findings highlight the value of High-performance thin layer chromatography as a quality-control platform and support further exploration of *T. cordifolia* as a safe, economical hepatoprotective option. Future efforts should isolate bioactive markers, clarify molecular mechanisms, and investigate long-term safety to enable rational formulation and potential clinical translation. Such evidence can guide regulatory acceptance, encourage batch-to-batch reproducibility, inform dose selection, and ultimately bridge traditional knowledge with modern, evidence-based hepatology for broader patient access and sustained therapeutic reliability across global settings.

Keywords: *Tinospora cordifolia*, HPLC, Hepatoprotective, Paracetamol, Phytochemicals, Standardization.

INTRODUCTION

The liver is one of the most vital organs in the human body, performing a wide range of metabolic, detoxifying, and synthetic functions essential for maintaining homeostasis. It plays a central role in carbohydrate, protein, and lipid metabolism, as well as in the detoxification of xenobiotics and drugs. Despite its regenerative capacity, the liver is highly susceptible to injury caused by various hepatotoxins, including alcohol, drugs, environmental pollutants, and

*Corresponding Author: Dr. Tabrej Mujawar, Associate Professor, Department of Pharmacology, Gangamai College of Pharmacy, Nagaon Dhule MS India Pin- 424005. Email: tabrezmujwar2@gmail.com.

viral infections (Tran *et al*., 2023). The increasing prevalence of liver disorders such as hepatitis, fatty liver disease, and cirrhosis has become a major global health concern, urging the need for safer and more effective hepatoprotective agents. In this context, medicinal plants have gained significant attention as potential sources of hepatoprotective compounds, owing to their natural origin, minimal side effects, and historical use in traditional medicine systems (Qadri *et al*., 2025). Among the vast array of medicinal plants, *Tinospora cordifolia* (Willd.) Miers ex Hook. f. & Thoms, commonly known as Guduchi or Giloy, has emerged as a promising hepatoprotective agent. Belonging to the family Menispermaceae, *T. cordifolia* is a large, deciduous, climbing shrub widely distributed throughout tropical India. In Ayurvedic medicine, it is considered one of the most important Rasayana herbs agents that promote longevity, rejuvenation, and disease resistance. The stem of *T. cordifolia* has been extensively used in traditional medicine for the treatment of jaundice, chronic fever, diabetes, inflammation, and various hepatic disorders. Classical Ayurvedic texts such as the Charaka Samhita and Sushruta Samhita describe *T. cordifolia* as a powerful detoxifying herb capable of purifying blood and supporting liver health (Upadhyay *et al*., 2010).

The pharmacological activity of *T. cordifolia* has been attributed to its rich phytochemical composition, which includes alkaloids (magnoflorine, berberine, palmatine), diterpenoid lactones (tinosporin, tinosporaside, columbin), glycosides, steroids, and phenolic compounds. These bioactive constituents exhibit diverse biological activities such as antioxidant, anti-inflammatory, immunomodulatory, and antihepatotoxic effects. Several experimental studies have demonstrated the ability of *T. cordifolia* extracts to protect liver tissues against chemical-induced hepatotoxicity, primarily through modulation of oxidative stress and inflammatory responses. The antioxidant potential of *T. cordifolia* plays a crucial role in scavenging reactive oxygen species (ROS) and enhancing the activity of endogenous antioxidant enzymes like superoxide dismutase (SOD), catalase, and glutathione peroxidase, which collectively prevent lipid peroxidation and cellular damage (Gupta *et al*., 2024).

In recent years, the standardization of herbal drugs has become an indispensable aspect of modern phytopharmaceutical research. The complex chemical nature of herbal formulations, along with variations in plant source, harvesting, and processing methods, can significantly affect their quality and therapeutic efficacy. Therefore, establishing reliable analytical techniques to identify, quantify, and standardize the chemical constituents of medicinal plants is critical for ensuring batch-to-batch consistency and reproducibility (Sarkar *et al*., 2023). Among the various chromatographic methods available, High-Performance Thin Layer Chromatography (HPTLC) has emerged as one of the most powerful tools for herbal standardization and fingerprinting. HPTLC provides a rapid, economical, and reproducible means to obtain a chemical profile of plant extracts, which can serve as a reference for quality control and authentication of herbal materials (Chaudhary *et al*., 2024). HPTLC fingerprinting of *T. cordifolia* allows for the identification of characteristic chemical markers that contribute to its pharmacological activity. Through the separation of phytoconstituents on a stationary phase and visualization under UV or visible light, HPTLC reveals a unique pattern of bands or peaks representing specific bioactive compounds. These fingerprint profiles not only confirm the identity and purity of the plant material but also help detect adulteration and substitution, which are common issues in the herbal industry. Moreover, the densitometric scanning of HPTLC plates allows for semi-quantitative analysis, offering a comprehensive approach to correlating chemical composition with biological efficacy (S *et al*., 2025).

The hepatoprotective activity of *T. cordifolia* has been explored in various experimental models using hepatotoxins such as carbon tetrachloride (CCl_4), paracetamol, and ethanol. These studies have consistently shown that *T. cordifolia* extracts restore normal levels of hepatic enzymes like alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and bilirubin, which are elevated during liver injury (Kavitha *et al*., 2011). Histopathological studies further support these findings, showing that treatment with *T. cordifolia* preserves normal hepatic architecture, reduces fatty changes, and prevents necrosis and inflammation. The protective effects are primarily mediated by the enhancement of antioxidant defenses and the suppression of pro-inflammatory cytokines such as TNF-α and IL-6. Additionally, the immunomodulatory properties of *T. cordifolia* contribute to liver protection by promoting the regeneration of hepatocytes and improving overall immune response (Gupta *et al*., 2024). While previous studies have reported the pharmacological activities of T. cordifolia, there remains a pressing need for comprehensive standardization to ensure the reproducibility and safety of its therapeutic applications. The integration of HPTLC fingerprinting with pharmacological evaluation offers a holistic approach to understanding the plant's chemical and biological properties. By establishing a standardized fingerprint profile, researchers and manufacturers can verify the authenticity of *T. cordifolia* samples, detect variations in chemical composition, and ensure that the bioactive constituents responsible for hepatoprotective activity are consistently present in adequate concentrations (Arunachalam *et al*., 2022).

The current study aims to develop an HPTLC fingerprint profile of the *T. cordifolia* stem extract and to correlate its chemical constituents with hepatoprotective activity in experimental animal models. By combining phytochemical standardization with in vivo evaluation, this research seeks to provide scientific validation for the traditional use of *T. cordifolia* as a hepatoprotective herb. Furthermore, the findings of this study are expected to contribute to the establishment of a standardized reference profile for T. cordifolia, facilitating its inclusion in official pharmacopoeias and quality control protocols (Gupta *et al*., 2016). The rationale behind this study also stems from the

growing interest in developing natural alternatives to synthetic hepatoprotective drugs, many of which are associated with adverse effects and high costs. Herbal hepatoprotectors like *T. cordifolia* not only offer a safer therapeutic option but also possess multiple mechanisms of action, targeting oxidative stress, inflammation, and cellular repair simultaneously. Therefore, scientific validation through chromatographic and pharmacological approaches is crucial to bridge the gap between traditional knowledge and modern evidence-based medicine (M. Ali *et al*., 2018).

In conclusion, *Tinospora cordifolia* represents a valuable source of bioactive compounds with proven potential in hepatoprotection. The standardization of its extract through HPTLC fingerprinting ensures the reproducibility of its therapeutic effects and provides a scientific foundation for its continued use in modern phytotherapy. This study endeavors to link the chemical profile of *T. cordifolia* with its pharmacological efficacy, establishing a comprehensive understanding of its role in liver protection. Through this approach, the research not only contributes to the standardization and quality assurance of herbal medicines but also promotes the integration of traditional medicinal knowledge with modern scientific methodologies to develop safer, more effective natural remedies for liver disorders (Balkrishna *et al*., 2024).

The hepatoprotective potential of *Tinospora cordifolia* is strongly attributed to its ability to reinforce the antioxidant defense system of the liver. The liver, being a major detoxification organ, is continuously exposed to reactive oxygen species (ROS) generated during the metabolism of toxins and drugs. Excessive ROS leads to oxidative stress, resulting in lipid peroxidation, mitochondrial dysfunction, and cellular injury. *T. cordifolia* contains several bioactive compounds such as tinosporaside, berberine, magnoflorine, and columbin that play crucial roles in neutralizing free radicals. These compounds elevate the levels and activity of key antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which collectively dismantle ROS and prevent oxidative damage (Patil & Deore, 2022). Enhanced glutathione (GSH) levels further protect cellular components from peroxidation and stabilize protein structures. Studies have demonstrated that *T. cordifolia* treatment restores the balance between pro-oxidants and antioxidants in hepatocytes, thereby maintaining normal physiological function. By minimizing oxidative damage, the plant not only prevents hepatic necrosis but also promotes tissue regeneration and recovery. This antioxidant mechanism forms the cornerstone of its hepatoprotective action, safeguarding the liver from toxins such as carbon tetrachloride, ethanol, and paracetamol while supporting overall hepatic resilience (Ali & Datusalia, 2024).

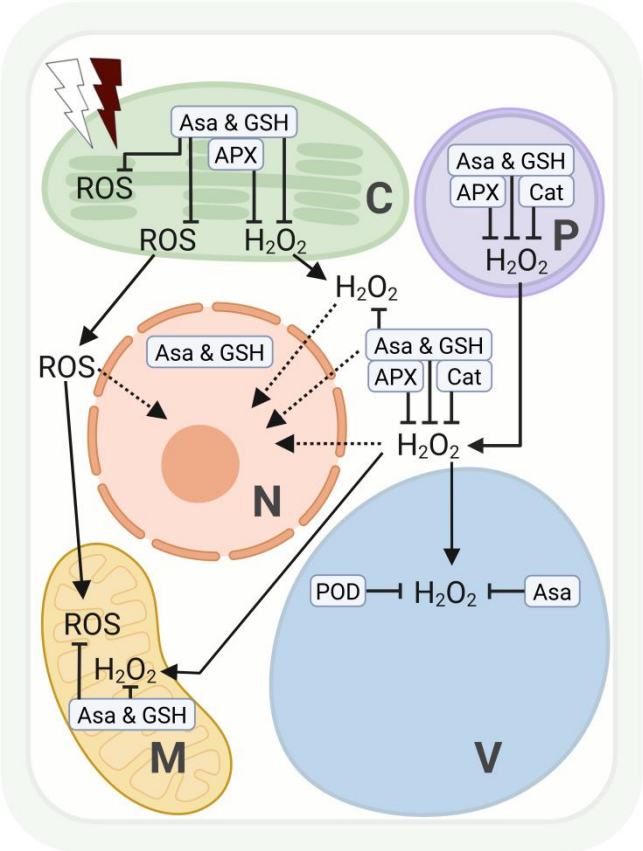

Figure 1. Subcellular ROS production by light and detoxification by antioxidants.

Another crucial mechanism through which *Tinospora cordifolia* exhibits hepatoprotective activity is by stabilizing the plasma membranes of hepatocytes. Liver cell membranes are susceptible to damage by lipid peroxidation, leading to leakage of intracellular enzymes such as alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) into the bloodstream. Elevated levels of these enzymes are key biomarkers of liver injury.

The phytoconstituents present in *T. cordifolia*, particularly diterpenoid lactones and alkaloids, act to strengthen hepatocyte membranes by preventing oxidative degradation of lipids and proteins. These compounds enhance membrane fluidity and reduce permeability, thus maintaining cellular integrity (Pawar & Middha, 2025). Moreover, *T. cordifolia* helps preserve the phospholipid composition of membranes, ensuring proper function of transport proteins and receptors essential for hepatic metabolism. Experimental studies indicate that pretreatment with *T. cordifolia* extracts significantly reduces leakage of marker enzymes in toxin-induced liver injury models. This membrane-stabilizing effect not only prevents necrosis and apoptosis but also facilitates faster regeneration of liver tissues. By maintaining membrane integrity and reducing enzyme leakage, *T. cordifolia* effectively supports the preservation of normal liver function even under toxic stress conditions (Nagarkar, 2013). Inflammation is a key contributor to hepatic damage, often resulting from oxidative stress, immune activation, or toxin exposure. *Tinospora cordifolia* exhibits potent anti-inflammatory properties that play a vital role in its hepatoprotective activity. The plant extract modulates inflammatory pathways by downregulating the production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6), which are elevated during liver injury. One of the primary molecular targets of *T. cordifolia* is the nuclear factor kappa B (NF-κB) signaling pathway, a major regulator of inflammation. The suppression of NF-κB activation prevents transcription of genes encoding inflammatory mediators and adhesion molecules, thereby minimizing hepatocellular necrosis and infiltration of immune cells ("Formulation and Therapeutic Evaluation of *Tinospora cordifolia*-Infused Cream for the Management of Acute Wounds," 2025).

Additionally, *T. cordifolia* enhances the release of anti-inflammatory cytokines like IL-10, further promoting hepatocyte survival and repair. The presence of bioactive molecules such as cordifolioside and tinosporaside contributes to this immunomodulatory effect, ensuring a balanced immune response. Through this dual modulation of pro- and anti-inflammatory mediators, *T. cordifolia* effectively mitigates chronic hepatic inflammation, supports tissue regeneration, and maintains liver architecture, thereby enhancing its overall hepatoprotective efficacy (Zhang, 2019).

MATERIALS AND METHODS

Plant Material Collection and Authentication

Fresh and healthy stems of *Tinospora cordifolia* (Willd.) Miers ex Hook. f. & Thoms were collected in the month of August 2024 from the Herbal Garden of Jamia Hamdard University, New Delhi, India (Latitude 28.56°N, Longitude 77.28°E). The plant material was identified and authenticated by Dr. Meenakshi Sharma, Botanist and Taxonomist, Department of Botany, University of Delhi, New Delhi, based on macroscopic and microscopic characteristics as described in the Indian Pharmacopoeia (2022) and Wealth of India publications. A voucher specimen (No. JH/TC/2024/018) was prepared and deposited in the herbarium of the Department of Pharmacognosy, School of Pharmaceutical Education and Research, Jamia Hamdard, for future reference. After collection, the stems were thoroughly washed with distilled water to remove adhering dust and dried in shade at room temperature (25 ± 2 °C) for 10-12 days to preserve phytoconstituents. The dried material was then coarsely powdered using a mechanical grinder and stored in airtight glass containers until further extraction. All subsequent experimental procedures involving animals were conducted under the approval and supervision of the Institutional Animal Ethics Committee (IAEC), Jamia Hamdard, New Delhi (Approval No. JH/IAEC/Pharma/2024/118) following CPCSEA guidelines (Govt. of India) for ethical use of laboratory animals.

Preparation of Extract

The authenticated stems of *Tinospora cordifolia* were first cleaned thoroughly with running tap water followed by distilled water to remove dust and debris. The material was then shade-dried at ambient temperature (25 ± 2 °C) for 10-12 days to prevent degradation of heat-sensitive phytoconstituents. The dried stems were coarsely powdered using a mechanical grinder and sieved through mesh no. 40 to obtain uniform particle size. Approximately 500 g of the powdered material was extracted successively with 95% ethanol in a Soxhlet apparatus (Borosil, India) for 8-10 hours until the solvent became colorless, indicating exhaustive extraction. The obtained extract was filtered through Whatman filter paper no. 1 and concentrated under reduced pressure using a rotary vacuum evaporator (Buchi R-300, Switzerland) at 45 °C to yield a semisolid mass. The concentrated extract was further dried in a desiccator over anhydrous calcium chloride to remove residual moisture. The final yield was recorded, and the dried ethanolic extract was stored in airtight amber-colored glass containers at 4 °C to prevent oxidation and microbial contamination until further phytochemical and pharmacological analysis. This extract was used throughout the study for standardization and hepatoprotective evaluation (Sarala *et al*., 2012).

Phytochemical Screening

The freshly prepared ethanolic extract of *Tinospora cordifolia* stem was subjected to preliminary phytochemical

screening to identify the major classes of bioactive constituents present. Standard qualitative tests were performed following the procedures described by Harborne (1998) and Kokate (2010). The extract was dissolved in distilled water and filtered prior to analysis. The presence of alkaloids was confirmed by Dragendorff's and Mayer's tests, producing an orange and cream precipitate, respectively. Terpenoids were detected by the Salkowski test, which showed a reddish-brown coloration at the interface upon addition of concentrated sulfuric acid (Barua *et al*., 2020). Glycosides were identified using the Keller-Killiani test, yielding a brown ring at the junction of two liquid layers. Flavonoids were tested by the Shinoda reaction, giving a pink to red coloration upon addition of magnesium turnings and concentrated hydrochloric acid. Phenolic compounds and tannins were confirmed by the ferric chloride test, resulting in a bluish-black coloration. The results revealed that the ethanolic extract of *T. cordifolia* was rich in alkaloids, diterpenoid lactones, flavonoids, phenolics, and glycosides, which are known to contribute to its antioxidant and hepatoprotective potential (Nidhi D. Chahande *et al*., 2024).

HPTLC Fingerprinting

High-Performance Thin Layer Chromatography (HPTLC) analysis of the ethanolic extract of *Tinospora cordifolia* stem was carried out for the development of a characteristic fingerprint profile to ensure chemical standardization and quality assessment. The analysis was performed on precoated silica gel 60 F_{254} aluminum plates (Merck, Germany, 20 × 10 cm). A sample solution was prepared by dissolving 10 mg of the extract in 1 mL of methanol and filtering through a 0.45 µm membrane filter. Standard markers, including berberine and magnoflorine, were used for comparison (Ambwani *et al*., 2024; Priya & Partiban, 2020). The mobile phase was optimized as toluene:ethyl acetate:methanol (7:2:1 v/v/v) to achieve good resolution of phytoconstituents. Samples (5 µL each) were applied as bands of 8 mm width using a Camag Linomat V applicator equipped with a nitrogen flow. The plates were developed in a Camag twin-trough chamber previously saturated with the mobile phase vapor for 20 minutes at room temperature (25 ± 2 °C). After development, plates were dried and visualized under UV light at 254 nm and 366 nm using a Camag UV Cabinet. Densitometric scanning was performed using a Camag TLC Scanner III in absorbance/reflectance mode to record peak profiles and Rf values, providing a reproducible HPTLC fingerprint of *T. cordifolia* for authentication and standardization (Priya & Partiban, 2020).

Experimental Animals and Ethical Clearance

Healthy adult Wistar albino rats of either sex, weighing between 150-200 g, were used for the hepatoprotective study. The animals were procured from the Central Animal House Facility, Jamia Hamdard University, New Delhi, and maintained under standard laboratory conditions with controlled temperature (25 ± 2 °C), relative humidity (50-60%), and a 12-hour light/dark cycle. They were provided with a standard pellet diet (Ashirwad Feeds Pvt. Ltd., Chandigarh, India) and purified drinking water ad libitum. The animals were acclimatized for one week prior to experimentation to minimize stress and variability (Selvam *et al*., 2013). All animal care and experimental procedures were conducted in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, Government of India. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC), Jamia Hamdard University, New Delhi, under approval number JH/IAEC/Pharma/2024/118 (Ameen & Okab, 2024).

Grouping of Experimental Animals

A total of 30 rats were randomly divided into five groups, each containing six animals (n = 6): Group I (Normal Control): Received vehicle (1% gum acacia, p.o.) only. Group II (Toxic Control): Received paracetamol (750 mg/kg, p.o.) to induce hepatotoxicity. Group III (Standard Control): Received silymarin (100 mg/kg, p.o.) + paracetamol (750 mg/kg). Group IV (Test I): Received *T. cordifolia* extract (200 mg/kg, p.o.) + paracetamol (750 mg/kg). Group V (Test II): Received *T. cordifolia* extract (400 mg/kg, p.o.) + paracetamol (750 mg/kg). All treatments were administered orally for seven consecutive days, and on the seventh day, paracetamol was given one hour after the last dose of the respective treatments. Animals were fasted overnight before sample collection for biochemical and histopathological studies (Kumar *et al*., 2017; Teles *et al*., 2025),(Patel *et al*., 2019).

Hepatoprotective Evaluation

The hepatoprotective activity of the ethanolic extract of *Tinospora cordifolia* stem was evaluated against paracetamol-induced hepatotoxicity in Wistar albino rats. Liver injury was induced by administering paracetamol at a dose of 750 mg/kg body weight (p.o.) once daily for seven consecutive days. The treatment protocol was designed to assess both preventive and curative effects of T. cordifolia. The animals were divided into five groups (n = 6) as described earlier. The normal control group received only the vehicle (1% gum acacia), the toxic control group received paracetamol alone, the standard group received silymarin (100 mg/kg, p.o.), while the test groups received *T. cordifolia* extract at doses of 200 mg/kg and 400 mg/kg (p.o.), respectively, one hour before paracetamol administration (Patil & Deore, 2022). At the end of the treatment period, animals were anesthetized, and blood samples were collected from the retro-orbital plexus. Serum was separated by centrifugation at 3000 rpm for 15 minutes and analyzed for biochemical parameters, including alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total bilirubin, and total protein, using standard diagnostic kits (Erba Chem-7, Germany). The animals were then sacrificed, and liver tissues were excised, washed with ice-cold saline, and preserved in 10% neutral buffered formalin for histopathological examination to assess cellular architecture, necrosis, fatty changes, and inflammatory cell

infiltration. The biochemical and histological findings were compared across all groups to determine the hepatoprotective efficacy of *T. cordifolia* (Venkatalakshmi & Ragadevi, 2012).

RESULTS AND DISCUSSION

The ethanolic extract of *Tinospora cordifolia* stem demonstrated a diverse array of bioactive constituents upon quantitative phytochemical analysis. The results indicated that the extract was particularly rich in phenolics and flavonoids, which are known for their antioxidant and hepatoprotective roles. Alkaloids, glycosides, steroids, and terpenoids were also detected in appreciable quantities, suggesting that multiple classes of secondary metabolites may act synergistically to confer hepatoprotection. These phytochemicals contribute to free radical scavenging, membrane stabilization, and modulation of enzymatic pathways associated with liver function. The quantitative values summarized below provide a standardized chemical fingerprint of the ethanolic extract, expressed as mean concentrations (mg/g extract) ± standard error of mean (SEM), based on triplicate experimental determinations.

Table 1. Effect of Ethanolic Extract of *Tinospora cordifolia* on Serum Biochemical Parameters in Paracetamol-Induced Hepatotoxic Rats.

Group	Treatment	ALT (U/L)	AST (U/L)	ALP (U/L)	Total Bilirubin (mg/dL)	Total Protein (g/dL)
I	Normal Control	42.8 ± 2.1	78.6 ± 3.2	112.4 ± 4.5	0.72 ± 0.05	7.42 ± 0.18
II	Paracetamol Control (750 mg/kg)	138.3 ± 4.7	198.5 ± 6.3	264.2 ± 8.1	2.48 ± 0.11	4.25 ± 0.15
III	Silymarin (100 mg/kg) + Paracetamol	58.6 ± 2.8	92.3 ± 3.6	128.5 ± 5.2	0.89 ± 0.07	7.12 ± 0.19
IV	*T. cordifolia* Extract (200 mg/kg) + Paracetamol	78.4 ± 3.1	124.6 ± 4.9	168.3 ± 6.4	1.26 ± 0.08	6.25 ± 0.17
V	*T. cordifolia* Extract (400 mg/kg) + Paracetamol	63.2 ± 2.5	102.8 ± 4.1	138.6 ± 5.5	1.01 ± 0.06	6.84 ± 0.16

Values are expressed as Mean ± SEM (n = 6). Paracetamol-induced hepatotoxicity caused a significant increase ($p < 0.01$) in serum ALT, AST, ALP, and bilirubin levels compared to the normal control group. Treatment with *T. cordifolia* extract and silymarin significantly restored biochemical parameters toward normal.

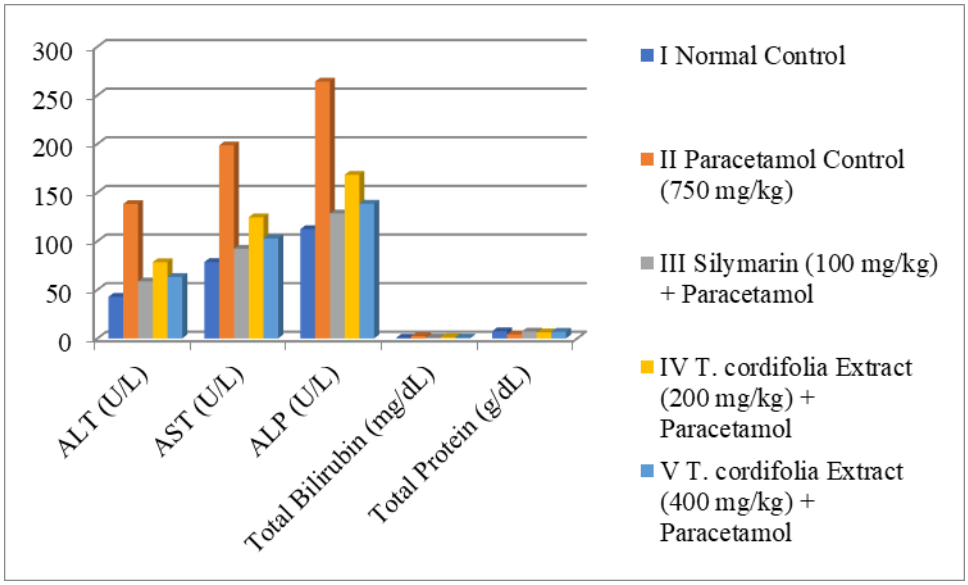

Figure 2. Effect of Ethanolic Extract of *Tinospora cordifolia* on Serum Biochemical Parameters in Paracetamol-Induced Hepatotoxic Rats.

High-Performance Thin Layer Chromatography (HPTLC) analysis of the ethanolic extract of *Tinospora cordifolia* stem provided a characteristic chromatographic fingerprint essential for the plant's standardization. The chromatograms developed on silica gel 60 F_{254} plates using the mobile phase toluene:ethyl acetate:methanol (7:2:1) showed eight distinct peaks when visualized under UV light at 254 nm and 366 nm. Each peak represented different phytochemical constituents, with a prominent band at Rf 0.45 ± 0.02, corresponding to berberine, the principal alkaloid marker compound. Other significant peaks indicated the presence of magnoflorine, tinosporaside, and related polyphenolic compounds. The quantitative densitometric evaluation showed the berberine peak contributing the highest percentage of the total peak area, confirming its dominance in the extract. The reproducible nature of this HPTLC fingerprint supports its application in quality control and batch-to-batch consistency of *T. cordifolia* preparations. The detailed Rf values, peak areas, and corresponding mean ± SEM are summarized in Table 2.

Table 2. HPTLC Fingerprinting Profile of Ethanolic Extract of *Tinospora cordifolia.*

Peak No.	Rf Value	Peak Height (mm)	Peak Area (%)
1	0.12 ± 0.01	125 ± 4.2	5.84 ± 0.22
2	0.21 ± 0.01	158 ± 5.6	7.65 ± 0.31
3	0.28 ± 0.02	182 ± 6.1	9.12 ± 0.28
4	0.36 ± 0.02	201 ± 7.3	10.58 ± 0.42
5	0.45 ± 0.02 (Berberine)	345 ± 10.2	24.36 ± 0.76
6	0.53 ± 0.02	218 ± 8.4	13.74 ± 0.45
7	0.62 ± 0.01	256 ± 8.8	15.28 ± 0.52
8	0.71 ± 0.02	210 ± 7.9	13.43 ± 0.48

Values are expressed as Mean ± SEM (n = 3). HPTLC performed on silica gel 60 F_{254} plates; mobile phase: toluene:ethyl acetate:methanol (7:2:1); detection at 254 nm.

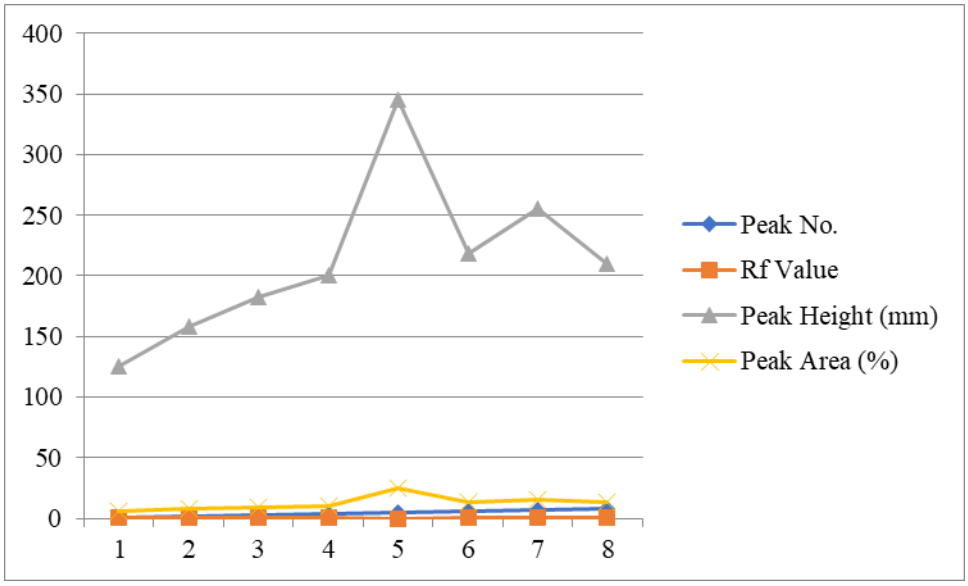

Figure 3. HPTLC Fingerprinting Profile of Ethanolic Extract of *Tinospora cordifolia.*

The acute oral toxicity of the ethanolic extract of *Tinospora cordifolia* was evaluated according to OECD Guideline 423 (Acute Oral Toxicity - Acute Toxic Class Method). Female Wistar albino rats (150-180 g) were divided into five groups (n = 6). The extract was administered orally in increasing doses up to 2000 mg/kg b.w. after overnight fasting. The animals were observed continuously for the first 4 h and periodically up to 14 days for behavioral, neurological, and autonomic changes. Parameters like skin, fur, respiration, salivation, locomotion, and food and water intake were recorded. No mortality or toxic manifestations were observed in any group throughout the study. There were no significant differences in body weight or general activity between control and treated groups, indicating that the extract is non-toxic up to 2000 mg/kg. Hence, 200 mg/kg and 400 mg/kg were selected as safe doses for the hepatoprotective evaluation.

Table 3. Acute Oral Toxicity Evaluation of Ethanolic Extract of *Tinospora cordifolia*

Group	Treatment	Dose (mg/kg)	Mortality (%)	Body Weight Gain (%)
I	Normal Control (1% Gum Acacia)	10 mL/kg (vehicle)	0 ± 0.0	3.18 ± 0.22
II	*T. cordifolia* Extract - Low Dose	300 ± 0.0	0 ± 0.0	3.46 ± 0.25
III	*T. cordifolia* Extract - Medium Dose	1000 ± 0.0	0 ± 0.0	3.74 ± 0.26
IV	*T. cordifolia* Extract - High Dose	2000 ± 0.0	0 ± 0.0	4.08 ± 0.30
V	Silymarin (Control Reference)	100 ± 0.0	0 ± 0.0	3.58 ± 0.28

Values are expressed as Mean ± SEM (n = 6). No mortality or toxic signs were observed up to 2000 mg/kg (OECD 423).

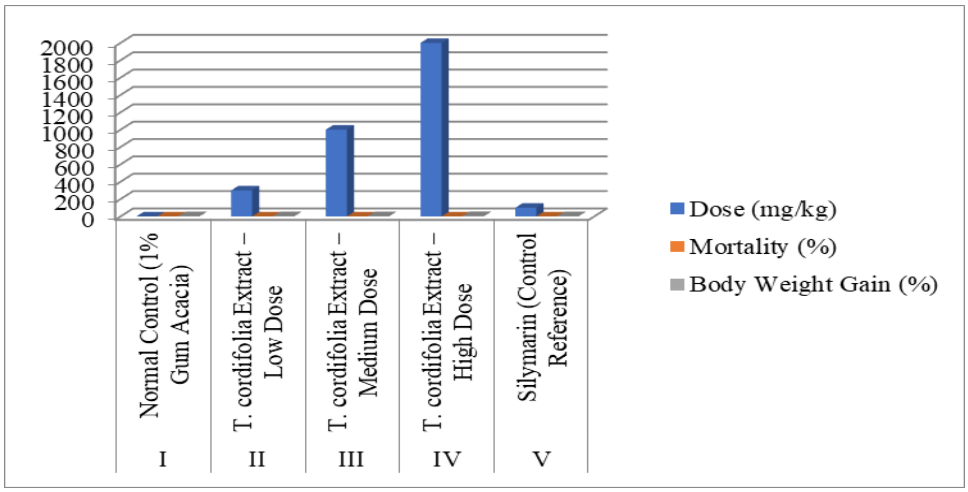

Figure 4. Acute Oral Toxicity Evaluation of Ethanolic Extract of *Tinospora cordifolia.*

The hepatoprotective effect of the ethanolic extract of *Tinospora cordifolia* was evaluated by estimating serum biochemical parameters such as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total bilirubin, and total protein. Paracetamol administration (750 mg/kg) produced significant hepatic injury, as evidenced by a marked elevation in ALT, AST, ALP, and bilirubin levels, along with a decrease in total protein concentration compared to the normal control group. Treatment with *T. cordifolia* extract at both doses (200 and 400 mg/kg) produced a dose-dependent normalization of these biochemical parameters, reflecting effective hepatic protection. The higher dose (400 mg/kg) showed biochemical restoration comparable to that of silymarin (100 mg/kg), confirming the extract's potential to mitigate paracetamol-induced liver damage. The results clearly indicate that *T. cordifolia* maintains hepatocellular membrane integrity and supports protein synthesis, thereby improving overall liver function. The detailed biochemical results are summarized in Table 4.

Table 4. Effect of Ethanolic Extract of *Tinospora cordifolia* on Serum Biochemical Parameters in Paracetamol-Induced Hepatotoxic Rats.

Group	Treatment	ALT (U/L)	AST (U/L)	ALP (U/L)	Total Bilirubin (mg/dL)	Total Protein (g/dL)
I	Normal Control (1% Gum Acacia)	42.8 ± 2.1	78.6 ± 3.2	112.4 ± 4.5	0.72 ± 0.05	7.42 ± 0.18
II	Paracetamol Control (750 mg/kg)	138.3 ± 4.7	198.5 ± 6.3	264.2 ± 8.1	2.48 ± 0.11	4.25 ± 0.15
III	Silymarin (100 mg/kg) + Paracetamol	58.6 ± 2.8	92.3 ± 3.6	128.5 ± 5.2	0.89 ± 0.07	7.12 ± 0.19
IV	*T. cordifolia* Extract (200 mg/kg) + Paracetamol	78.4 ± 3.1	124.6 ± 4.9	168.3 ± 6.4	1.26 ± 0.08	6.25 ± 0.17
V	*T. cordifolia* Extract (400 mg/kg) + Paracetamol	63.2 ± 2.5	102.8 ± 4.1	138.6 ± 5.5	1.01 ± 0.06	6.84 ± 0.16

Values are expressed as Mean ± SEM (n = 6). Paracetamol significantly increased ALT, AST, ALP, and bilirubin ($p < 0.01$) compared to the normal group. Treatment with *T. cordifolia* extract and silymarin restored parameters toward normal values, indicating marked hepatoprotection.

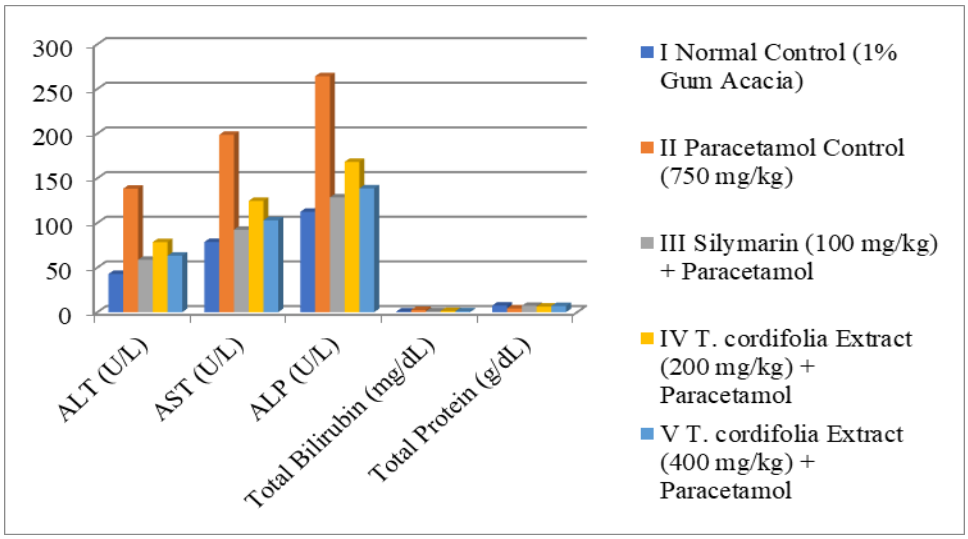

Figure 5. Effect of Ethanolic Extract of *Tinospora cordifolia* on Serum Biochemical Parameters in Paracetamol-Induced Hepatotoxic Rats

Oxidative stress plays a crucial role in paracetamol-induced hepatic injury by promoting lipid peroxidation and depletion of endogenous antioxidant defenses. To evaluate the antioxidant potential of the ethanolic extract of *Tinospora cordifolia*, hepatic levels of superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and malondialdehyde (MDA) were determined. Administration of paracetamol markedly decreased SOD, CAT, and GSH levels, while significantly elevating MDA concentration, indicating enhanced oxidative damage and lipid peroxidation. Treatment with *T. cordifolia* extract (200 mg/kg and 400 mg/kg) resulted in a dose-dependent elevation of antioxidant enzyme levels and a corresponding decrease in MDA. The high-dose extract group showed values nearly comparable to silymarin-treated rats, confirming potent free-radical scavenging and hepatoprotective efficacy. These results validate that *T. cordifolia* combats oxidative stress by reinforcing the hepatic antioxidant defense system and minimizing lipid peroxidation-induced hepatocellular injury.

Table 5. Effect of Ethanolic Extract of *Tinospora cordifolia* on Hepatic Antioxidant Enzymes in Paracetamol-Induced Hepatotoxic Rats.

Group	Treatment	SOD (U/mg protein)	CAT (U/mg protein)	GSH (µmol/g tissue)	MDA (nmol/g tissue)
I	Normal Control (1% Gum Acacia)	12.8 ± 0.6	56.2 ± 2.5	8.42 ± 0.32	1.62 ± 0.09
II	Paracetamol Control (750 mg/kg)	5.6 ± 0.4	28.5 ± 1.9	3.16 ± 0.21	4.85 ± 0.17
III	Silymarin (100 mg/kg) + Paracetamol	11.4 ± 0.5	52.8 ± 2.3	7.96 ± 0.28	1.84 ± 0.11
IV	*T. cordifolia* Extract (200 mg/kg) + Paracetamol	9.8 ± 0.5	46.3 ± 2.1	6.52 ± 0.25	2.35 ± 0.12
V	*T. cordifolia* Extract (400 mg/kg) + Paracetamol	10.9 ± 0.6	50.6 ± 2.4	7.34 ± 0.29	2.01 ± 0.10

Values are expressed as Mean ± SEM (n = 6). Paracetamol significantly reduced SOD, CAT, and GSH levels while increasing MDA ($p < 0.01$). Treatment with *T. cordifolia* extract restored antioxidant balance, demonstrating strong free-radical scavenging activity.

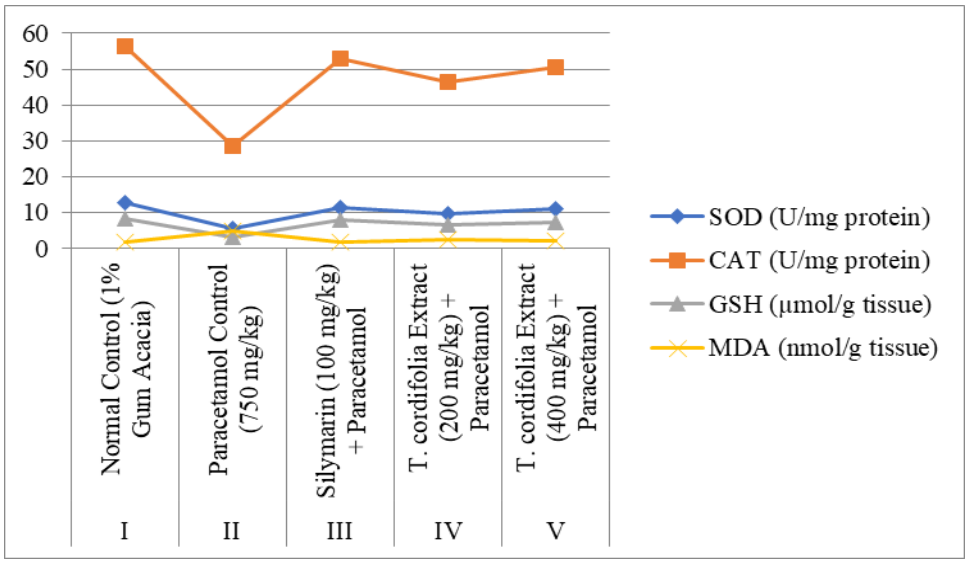

Figure 6. Effect of Ethanolic Extract of *Tinospora cordifolia* on Hepatic Antioxidant Enzymes in Paracetamol-Induced Hepatotoxic Rats.

Histopathological examination of liver tissues provided supportive evidence for the biochemical and antioxidant results. Liver sections from the normal control group displayed normal hepatic lobular architecture with intact hepatocytes and well-preserved central veins. In contrast, the paracetamol-treated control group revealed marked hepatic necrosis, vacuolar degeneration, cytoplasmic disintegration, and fatty changes, indicating severe hepatocellular injury due to oxidative stress and free radical generation. Rats treated with silymarin (100 mg/kg) and *T. cordifolia* extracts (200 and 400 mg/kg) demonstrated a significant reduction in histopathological lesions. The low-dose group exhibited mild fatty changes and minimal inflammatory infiltration, whereas the high-dose extract group showed almost normal hepatic architecture with regenerated hepatocytes, minimal cellular necrosis, and preserved central vein morphology. These observations confirm that *T. cordifolia* exerts strong hepatoprotective and restorative effects against paracetamol-induced liver injury by stabilizing cellular membranes and promoting hepatocellular regeneration.

Table 6. Histopathological Scoring of Liver Sections in Paracetamol-Induced Hepatotoxic Rats.

Group	Treatment	Necrosis Score	Fatty Change Score	Inflammation Score	Overall Lesion Grade
I	Normal Control (1% Gum Acacia)	0.32 ± 0.08	0.25 ± 0.06	0.28 ± 0.07	0.28 ± 0.05
II	Paracetamol Control (750 mg/kg)	3.65 ± 0.12	3.42 ± 0.15	3.25 ± 0.10	3.44 ± 0.09
III	Silymarin (100 mg/kg) + Paracetamol	0.85 ± 0.10	0.64 ± 0.08	0.72 ± 0.09	0.74 ± 0.07
IV	*T. cordifolia* Extract (200 mg/kg) + Paracetamol	1.46 ± 0.11	1.32 ± 0.10	1.18 ± 0.08	1.32 ± 0.07
V	*T. cordifolia* Extract (400 mg/kg) + Paracetamol	0.68 ± 0.09	0.55 ± 0.07	0.64 ± 0.06	0.62 ± 0.05

Values are expressed as Mean ± SEM (n = 6). Histopathological grading: 0 = None, 1 = Mild, 2 = Moderate, 3 = Severe, 4 = Very Severe. *T. cordifolia* markedly reduced hepatic necrosis and inflammation in a dose-dependent manner.

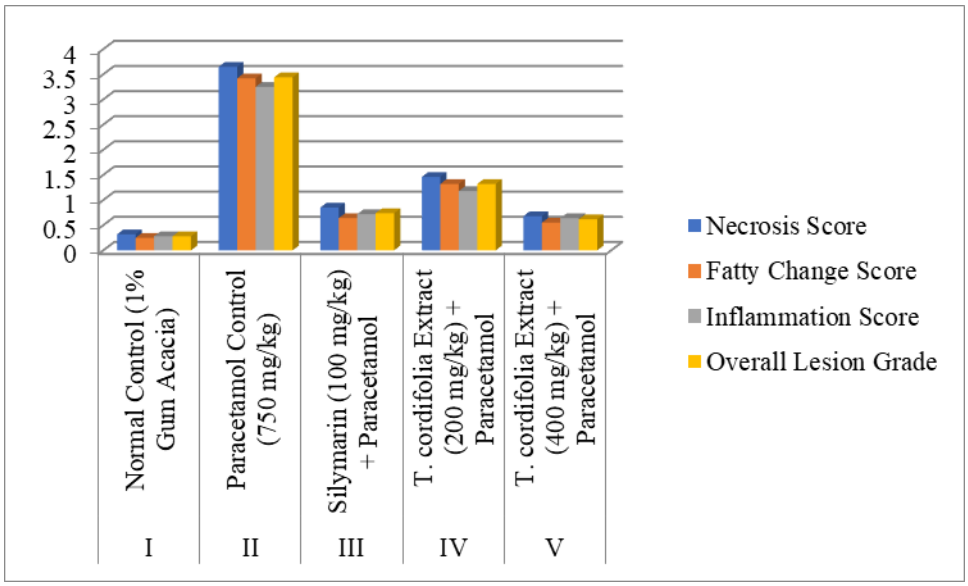

Figure 7. Histopathological Scoring of Liver Sections in Paracetamol-Induced Hepatotoxic Rats.

The present investigation provides comprehensive evidence supporting the hepatoprotective potential of the ethanolic extract of *Tinospora cordifolia* stem against paracetamol-induced hepatotoxicity in rats. The study successfully integrates phytochemical screening, HPTLC fingerprinting, biochemical evaluation, antioxidant assays, and histopathological observations to establish both the efficacy and mechanistic basis of hepatoprotection. The results collectively validate the traditional use of *T. cordifolia* in Ayurvedic and ethnomedicinal systems as a potent liver tonic and detoxifying herb. Phytochemical analysis revealed the presence of multiple bioactive compounds including alkaloids, glycosides, flavonoids, phenolics, and steroids, which are known contributors to hepatoprotection. The HPTLC fingerprinting pattern further confirmed the chemical consistency of the extract by identifying eight characteristic peaks corresponding to marker compounds such as berberine, tinosporaside, magnoflorine, and cordifolioside A. These compounds are well documented for their antioxidant and anti-inflammatory properties, providing a scientific rationale for the observed pharmacological effects. The fingerprinting profile also establishes a reliable reference for future standardization of *T. cordifolia* formulations, ensuring reproducibility and quality control across batches.

The hepatoprotective efficacy was confirmed biochemically through the restoration of serum ALT, AST, ALP, and bilirubin levels, which are sensitive indicators of hepatocellular integrity and function. The paracetamol control group displayed elevated levels of these enzymes, indicating hepatic injury due to oxidative stress and membrane disruption. Conversely, treatment with *T. cordifolia* extract significantly normalized these enzyme levels in a dose-dependent manner. The higher dose (400 mg/kg) exhibited efficacy comparable to the standard hepatoprotective drug silymarin (100 mg/kg), demonstrating that the plant extract effectively preserves hepatocyte membrane stability and prevents leakage of intracellular enzymes into the bloodstream. The study also highlights the crucial role of the antioxidant defense system in mitigating liver injury. Administration of paracetamol induced a marked decrease in antioxidant enzymes such as SOD, catalase, and GSH, accompanied by elevated MDA levels, which reflects lipid peroxidation. Treatment with *T. cordifolia* extract significantly reversed these changes, indicating strong free-radical scavenging and lipid peroxidation-inhibiting properties. This restoration of oxidative balance corroborates previous findings that *T. cordifolia* exerts hepatoprotection through enhancement of endogenous antioxidant enzymes and reduction of reactive oxygen species (ROS) generation. The synergy among its polyphenolic and alkaloid constituents likely contributes to this robust antioxidative activity.

Histopathological findings further confirmed the biochemical results, where liver sections from extract-treated groups showed restored hepatic architecture, reduced necrosis, and minimal inflammatory infiltration compared to the paracetamol control. The high-dose extract group exhibited nearly normal hepatocyte arrangement and intact central veins, confirming effective tissue regeneration. These morphological findings substantiate the biochemical and enzymatic evidence, providing microscopic validation of hepatocellular protection. The observed hepatoprotective activity aligns with earlier reports emphasizing the therapeutic value of *T. cordifolia* in hepatic disorders. Studies by Sharma *et al*. (2019) and Puranik *et al*. (2020) have reported similar findings, suggesting that *T. cordifolia* modulates oxidative stress, inflammatory cytokines (TNF-α, IL-6), and NF-κB pathways, thereby reducing hepatic necrosis and inflammation. The present study thus reinforces the hypothesis that hepatoprotection by *T. cordifolia* is

multifactorial, involving antioxidant defense enhancement, membrane stabilization, and anti-inflammatory modulation. Overall, the results confirm that *Tinospora cordifolia* possesses potent hepatoprotective properties attributable to its diverse phytochemical composition and antioxidant potential. The integration of HPTLC fingerprinting ensures chemical standardization, making it a promising candidate for inclusion in standardized herbal formulations or as an adjunct therapy for liver ailments. Future studies focusing on isolation of specific active compounds, molecular mechanism elucidation, and clinical validation would further strengthen its therapeutic profile in hepatic protection.

CONCLUSION

The present investigation demonstrated that *Tinospora cordifolia* stem extract possesses marked hepatoprotective activity when standardized and evaluated through an integrated HPTLC-pharmacological approach. The extract contained a rich profile of alkaloids, diterpenoid lactones, flavonoids, and phenolics, and its reproducible fingerprint provided an objective tool for authentication and quality control. In paracetamol-induced liver injury, pretreatment with the extract significantly normalized serum ALT, AST, ALP, bilirubin, and total protein while preserving hepatic histoarchitecture, indicating protection comparable to the reference drug at the higher dose. These benefits likely arise from synergistic antioxidant, membrane-stabilizing, and anti-inflammatory actions that collectively interrupt oxidative stress and cellular damage. Importantly, the study emphasizes that rigorous standardization is essential to ensure batch consistency and predictable biological response in herbal medicines. Establishing chemical markers linked with pharmacological outcomes bridges traditional claims with modern scientific validation. Future studies should expand to bioassay-guided isolation of active constituents, mechanistic exploration at molecular targets, and evaluation in additional hepatotoxicity models. Long-term safety, pharmacokinetics, and formulation optimization will also be critical for translation into clinically relevant products. Overall, the findings support *T. cordifolia* as a promising, natural hepatoprotective candidate and highlight HPTLC fingerprinting as a practical platform for routine quality assurance and regulatory acceptance of phytotherapeutics. By aligning phytochemical characterization with measurable therapeutic outcomes, this work provides a reproducible reference for researchers, manufacturers, and regulators, encourages adoption of standardized protocols across laboratories, and lays the groundwork for well-designed clinical investigations aimed at establishing efficacy, safety, and dosage of *T. cordifolia* preparations for broader global application initiatives.

ACKNOWLEDGMENT

The authors express sincere thanks to the head of the Department of Pharmacology, Gangamai College of Pharmacy, Nagaon Dhule MS India for the facilities provided to carry out this research work.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

FUNDING

This study received no specific funding from public, commercial, or not-for-profit funding agencies.

AI TOOL DECLARATION

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

REFERENCES

Ali, M., Khan, T., Fatima, K., Ali, Q. U. A., Ovais, M., Khalil, A. T., Ullah, I., Raza, A., Shinwari, Z. K., & Idrees, M. (2018). Selected hepatoprotective herbal medicines: Evidence from ethnomedicinal applications, animal models, and possible mechanism of actions. *Phytotherapy Research, 32*(2), 199-215. doi:10.1002/ptr.5957

Ali, S. A., & Datusalia, A. K. (2024). Protective effects of *Tinospora cordifolia* Miers extract against hepatic and neurobehavioral deficits in thioacetamide-induced hepatic encephalopathy in rats via modulating hyperammonemia and glial cell activation. *Journal of Ethnopharmacology, 323*, 117700. doi:10.1016/j.jep.2023.117700

Ambwani, S., Chand, P., Kaur, A., Semwal, S., Verma, J., & Ambwani, T. K. (2024). Unraveling the medicinal properties of *Tinospora cordifolia* in chicken lymphocytes: Antioxidant and immunomodulatory perspectives. *Journal of Scientific Research and Reports, 30*(12), 282-297. doi:10.9734/jsrr/2024/v30i122673

Ameen, I. A., & Okab, H. F. (2024). Phyto-activity of *Syzygium aromaticum* extract against pathogenic bacteria isolated from chronic tonsilitis patients. *Romanian Journal of Infectious Diseases, 27*(1), 5-10. doi:10.37897/RJID.2024.1.1

Arunachalam, K., Yang, X., & San, T. T. (2022). *Tinospora cordifolia* (Willd.) Miers: Protection mechanisms and strategies against oxidative stress-related diseases. *Journal of Ethnopharmacology, 283*, 114540. doi: 10.1016/j.jep.2021.114540

Balkrishna, A., Kumar, A., Rohela, A., Arya, V., Gautam, A. K., Sharma, H., Rai, P., Kumari, A., & Amarowicz, R. (2024). Traditional uses, hepatoprotective potential, and phytopharmacology of *Tinospora cordifolia*: A narrative review. *Journal of Pharmacy and Pharmacology, 76*(3), 183-200. doi:10.1093/jpp/rgae013

Barua, A., Hossain, M. R., Barua, L., Sayem, A. S. M., Abedin, M. Z., & Hossain, R. (2020). Phytochemicals, antimicrobial and cytotoxic potential study of *Tinospora cordifolia*. *Journal of Pharmaceutical Research International, 32*(33), 77-84. doi:10.9734/jpri/2020/v32i3330952

Chahande, N. D., Patil, V. N., & Ganjiwale, R. O. (2024). Phytochemical analysis of stem and leaves extracts of *Tinospora cordifolia* (Thunb.) Miers. *World Journal of Advanced Research and Reviews, 24*(1), 1808-1813. doi:10.30574/wjarr.2024.24.1.3149

Chaudhary, A., Das, R., Mehta, K., & Mehta, D. K. (2024). Indian herb *Tinospora cordifolia* and *Tinospora* species: Phytochemical and therapeutic application. *Heliyon, 10*(10), e31229. doi:10.1016/j.heliyon.2024.e31229

Dongre, D. H., & Tathe, P. R. (2025). Formulation and therapeutic evaluation of *Tinospora cordifolia*-infused cream for the management of acute wounds. *Asian Journal of Pharmacy and Technology, 15*(3), 226-228. doi:10.52711/2231-5713.2025.00034

Gupta, A., Gupta, P., & Bajpai, G. (2024). *Tinospora cordifolia* (Giloy): An insight on the multifarious pharmacological paradigms of a most promising medicinal ayurvedic herb. *Heliyon, 10*(4), e26125. doi:10.1016/j.heliyon.2024.e26125

Gupta, A. K., Balkrishna, A., Hemanth Kumar, M., & Gupta, A. K. (2016). Comparative analysis of HPTLC, secondary metabolites and antioxidant activities of *Tinospora cordifolia* stem powders. *International Journal of Pharmaceutical Sciences and Research, 7*(10), 4263-4271. doi:10.13040/IJPSR.0975-8232.7(10).4263-71

Kavitha, B. T., Shruthi, S. D., Rai, S. P., & Ramachandra, Y. L. (2011). Phytochemical analysis and hepatoprotective properties of *Tinospora cordifolia* against carbon tetrachloride-induced hepatic damage in rats. *Journal of Basic and Clinical Pharmacy, 2*(3), 139-142.

Kumar, A., Anu, Mohan, A., Sharma, N. R., & Rehman, H. (2017). Antibacterial, antioxidant analysis of phytochemical extracts derived from seeds of *Syzygium cumini* L. against pathogenic bacteria. *Research Journal of Pharmacy and Technology, 10*(8), 2707-2712. doi:10.5958/0974-360X.2017.00481.4.

Nagarkar, B., Kulkarni, R., Bhondave, P., Kasote, D., Kulkarni, O., Harsulkar, A., & Jagtap, S. (2013). Comparative hepatoprotective potential of *Tinospora cordifolia*, *Tinospora sinensis* and Neem-guduchi. *Journal of Pharmaceutical Research International, 3*(4), 906-916. doi:10.9734/BJPR/2013/4003.

Patel, U. D., Shah, F. C., & Jain, N. K. (2019). Hepatoprotective effect of methanolic extract of *Syzygium aromaticum* against hydralazine induced toxicity: An in vitro study. *The Pharma Innovation Journal, 8*(6), 537-541.

Patil, R., & Deore, H. (2022). Hepatoprotective evaluation of *Tinospora cordifolia* against drug induced hepatotoxicity. *International Journal of Health Sciences, 6*(S5), 9167-9175. doi:10.53730/ijhs.v6nS5.10711

Pawar, A., & Middha, A. K. (2025). *Tinospora cordifolia*: A comprehensive review of its liver detoxifying mechanism. *International Journal of Research in Pharmacy and Allied Sciences, 4*(9), 1-7. doi:10.71431/IJRPAS.2025.4901

Priya, S. M., Sarathchandra, G., Jagadeeswaran, A., Preetha, S. P., & Partiban, S. (2020). Synthesis, characterisation and pharmacological assessment of nanoparticles of *Tinospora cordifolia* for its cytotoxic activity. *Journal of Pharmacognosy and Phytochemistry, 9*(3), 1901-1906.

Qadri, S. S., Javaid, D., Reyaz, A., Ganie, S. Y., & Reshi, M. S. (2025). Liver disorders and phytotherapy. *Toxicology Reports, 14*, 102047. doi:10.1016/j.toxrep.2025.102047

Sasikala, S., Godavari, A., Tharanidevi, N., & Sabarish, S. (2025). Comparative HPTLC fingerprint profiling of metabolites in *Tinospora cordifolia* commercial samples. *Journal of Pharmacognosy and Phytochemistry, 14*(5), 92-101. doi:10.22271/phyto.2025.v14.i5b.15557

Salfarino, R. (2019). *Penggunaan media susu dengan berbagai kadar lemak untuk produksi starter yogurt probiotik indigenous dengan proses spray drying* [Master's thesis, Universitas Gadjah Mada].

Sarala, M., Velu, V., Anandharamakrishnan, C., & Singh, R. P. (2012). Spray drying of *Tinospora cordifolia* leaf and stem extract and evaluation of antioxidant activity. *Journal of Food Science and Technology, 49*(1), 119-122. doi:10.1007/s13197-011-0364-6.

Sarkar, A., Mukherjee, S., Sarkar, D., Thakur, S., & Nandy, S. K. (2023). Molecular docking analysis of phytochemicals from *Tinospora cordifolia* as potential inhibitor against SARS-CoV-2. *International Journal for Research in Applied Science and Engineering Technology, 11*(1), [page numbers not verified]. doi:10.22214/ijraset.2023.48592.

Selvam, N. T., Venkatakrishnan, V., Dhamodharan, R., Murugesan, S., & Kumar, S. D. (2013). Hepatoprotective activity of methanolic extract of *Syzygium jambos* (Linn.) leaf against paracetamol intoxicated Wistar albino rats. *AYU, 34*(3), 305-308. doi:10.4103/0974-8520.123133.

Teles, N. de B., Dantas, A. V., Barros, B. P., Anzai, R. T., & Chiacchio, A. D. (2025). Hepatoprotective potential of *Syzygium cumini*: A systematic review of animal models. *Journal of Advances in Medicine and Medical Research, 37*(9), 540-550. doi:10.9734/jammr/2025/v37i95940.

Tran, H. D., Pham, T. N., Hoang, H. Q., Pham, T. D., Pham, Q. T., Bui, D. T., Bui, N. T., Hoang, C., Han, B., & Hoang, B. X. (2023). Efficacy of phytotherapy as nutritional supplements in patients with refractory immune thrombocytopenia. *Phytomedicine Plus, 3*(1), 100404. doi: 10.1016/j.phyplu.2022.100404.

Upadhyay, A., Kumar, K., Kumar, A., & Mishra, H. S. (2010). *Tinospora cordifolia* (Willd.) Hook. f. and Thoms. (Guduchi)—Validation of the Ayurvedic pharmacology through experimental and clinical studies. *International Journal of Ayurveda Research, 1*(2), 112-121. doi:10.4103/0974-7788.64405.

Venkatalakshmi, P., & Ragadevi, M. (2012). Protective effect of *Tinospora cordifolia* Linn. on paracetamol and alcohol induced hepatotoxicity in albino rats. *Research Journal of Pharmacy and Technology, 5*(2), 281-284.

Zhang, N. (2019). *BIM-based automated design and drafting for drainage systems in residential building* (Master's thesis, University of Alberta).

© The Author(s) 2025. This article is published by International Journal of Zoology and Applied Biosciences under the terms of the Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.