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Research Article

In Vitro Evaluation of the Synergistic Hepatoprotective Potential of *Ficus benghalensis* and *Tinospora cordifolia* Against Drug-Induced Hepatotoxicity in Hepg2 Cells

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ABSTRACT

Background: Drug-induced hepatotoxicity is a major cause of liver injury and is associated with oxidative stress, membrane damage, and depletion of endogenous antioxidant defenses. *Ficus benghalensis* and *Tinospora cordifolia* possess diverse phytoconstituents and have been traditionally used for various liver-related disorders. However, their combined hepatoprotective potential against drug-induced hepatotoxicity requires further investigation. The present study evaluated the individual and combined hepatoprotective effects of these plants in HepG2 cells. **Methodology:** Methanolic extracts of *F. benghalensis* and *T. cordifolia* were prepared and subjected to preliminary phytochemical screening. HepG2 cells were exposed to a hepatotoxic drug to establish an in vitro hepatotoxicity model. Cytotoxicity of both extracts was assessed by the MTT assay, and 200 µg/mL was selected as the treatment concentration. Hepatoprotective activity was evaluated using cell viability, morphological changes, LDH release, ROS, MDA, GSH, SOD, CAT, and total protein levels. **Results:** The hepatotoxic drug reduced cell viability to $51.26 \pm 2.91\%$, whereas treatment with *F. benghalensis* and *T. cordifolia* increased viability to $72.84 \pm 2.63\%$ and $76.52 \pm 2.47\%$, respectively. Combined treatment produced $88.67 \pm 2.21\%$ viability and 76.17% hepatoprotection, compared with 42.54% and 51.06% for the individual extracts. The combination reduced LDH, ROS, and MDA levels while restoring GSH, SOD, CAT, and total protein levels. **Conclusion:** The combined extracts demonstrated enhanced hepatoprotective activity compared with individual treatments, suggesting complementary or potential synergistic effects. However, formal concentration-response synergy analysis and further molecular and in vivo studies are required to confirm synergism.

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INTRODCUTION:

1.1. Overview and Global Burden of Liver Diseases

The liver is one of the major metabolic and detoxifying organs of the human body and performs essential functions including nutrient metabolism, synthesis of plasma proteins, bile production, regulation of lipid and carbohydrate metabolism, and biotransformation of endogenous and exogenous substances. Liver diseases comprise a broad spectrum of acute and chronic disorders that impair these functions and may progress from mild hepatic injury to fibrosis, cirrhosis, liver failure, and hepatocellular carcinoma. Major categories include viral hepatitis, alcohol-associated liver disease, metabolic dysfunction-associated steatotic liver disease (MASLD), autoimmune and genetic liver disorders, and drug- or toxin-induced liver injury. The global burden of liver disease remains substantial. Recent Global Burden of Disease (GBD) 2021 estimates reported approximately 309.72 million new liver disease cases, 1.98 million deaths, and 63.53 million disability-adjusted life-years (DALYs) worldwide in 2021.

The epidemiology of liver disease has changed considerably with changing lifestyles, metabolic risk factors, alcohol consumption, persistent viral infections, and increasing exposure to therapeutic and environmental chemicals. In 2021, cirrhosis and other chronic liver diseases alone were estimated to account for approximately 58.4 million incident cases, 1.43 million deaths, and 46.4 million DALYs globally. Between 2010 and 2021, the number of incident cases increased by approximately 18%, while deaths increased by about 7%, indicating that liver disease continues to represent a major public-health challenge despite improvements in age-standardized mortality. Metabolic dysfunction-associated steatohepatitis (MASH) was particularly important, contributing approximately 48.3 million incident cases of cirrhosis and other chronic liver diseases in 2021 and showing a substantial increase in incidence, deaths, and DALYs during the study period.

Viral hepatitis continues to contribute substantially to the global burden, particularly through chronic hepatitis B and C infections that can progress to cirrhosis and liver cancer. According to recent WHO estimates,

approximately 287 million people were living with chronic hepatitis B or C in 2024, and viral hepatitis B- and C-related cirrhosis and liver cancer resulted in approximately 1.3 million deaths during the same year. Liver cancer itself also represents a major global health problem, with IARC estimating approximately 850,000 new liver cancer cases and more than 730,000 deaths in 2024. In India, cirrhosis remains among the important causes of mortality, with WHO reporting a cirrhosis mortality rate of approximately 21.8 deaths per 100,000 population in 2023. These epidemiological trends highlight the continuing need for effective strategies to prevent hepatic injury and identify hepatoprotective interventions.

1.1.1. Drug-Induced Hepatotoxicity and Its Mechanisms

Drug-induced liver injury (DILI) refers to hepatic damage caused by exposure to therapeutic drugs, their metabolites, or other medicinal substances. It represents an important form of liver injury because the liver is the primary organ responsible for the metabolism and detoxification of many xenobiotics. DILI may present as hepatocellular, cholestatic, or mixed liver injury and may occur through intrinsic, dose-dependent mechanisms or through idiosyncratic mechanisms that are less predictable. Epidemiological studies indicate that DILI is relatively uncommon at the population level but remains clinically important because of its potential severity. A systematic review and meta-analysis of population-based studies estimated an overall DILI incidence of approximately 4.94 cases per 100,000 person-years, with considerably higher incidence reported in Asian populations (17.82 cases per 100,000 person-years).

The development of drug-induced hepatotoxicity involves several interconnected cellular and molecular mechanisms. Bioactivation of certain drugs can generate reactive metabolites and excessive reactive oxygen species (ROS), resulting in oxidative stress and depletion of endogenous antioxidants. Excessive ROS can damage cellular proteins, membrane lipids, and DNA, while mitochondrial dysfunction can impair ATP production and further increase oxidative stress. In addition, activation of inflammatory pathways and immune responses can amplify hepatocyte injury. Severe



cellular stress may ultimately activate apoptotic and necrotic pathways, resulting in loss of viable hepatocytes. Current evidence identifies mitochondrial dysfunction, increased ROS generation, apoptosis, necrosis, and immune-mediated injury among the important mechanisms involved in DILI.

Several widely used therapeutic agents have been associated with drug-induced liver injury. Acetaminophen (paracetamol) is a well-established example of intrinsic, dose-dependent hepatotoxicity, particularly following excessive exposure, whereas many antibiotics, anti-tuberculosis drugs, anticonvulsants, and other therapeutic agents can produce idiosyncratic liver injury. Recent clinical evidence indicates that antibiotics remain major contributors to DILI in Western countries, with agents such as amoxicillin-clavulanate frequently implicated. Importantly, DILI may range from transient biochemical abnormalities to severe acute liver failure, and a proportion of affected individuals can develop persistent or chronic liver injury. Therefore, understanding the cellular mechanisms underlying drug-induced hepatotoxicity and identifying compounds capable of reducing oxidative, inflammatory, mitochondrial, and apoptotic damage is important for the development of potential hepatoprotective strategies.

1.2. *Ficus benghalensis*

Ficus benghalensis L., commonly known as the banyan tree or Indian banyan, belongs to the family Moraceae and is widely distributed throughout the Indian subcontinent and other tropical regions. It is a large, perennial, evergreen to semi-evergreen tree characterized by a broad crown and numerous aerial prop roots that develop from its branches and eventually reach the soil. The leaves are simple, alternate, broad, and leathery, while the flowers are enclosed within specialized inflorescences known as syconia. Different parts of the plant, including the bark, leaves, roots, aerial roots, fruits, and latex, have been investigated for their medicinal properties. The extensive use of different plant parts has made *F. benghalensis* an important species in traditional systems of medicine.

The plant has a long history of use in Ayurvedic and other traditional medicinal practices. Preparations

obtained from the bark, roots, leaves, and latex have traditionally been used for conditions including diarrhoea, dysentery, diabetes, inflammation, wounds, skin disorders, and rheumatic complaints. The latex has particularly been associated with traditional applications in wound management, while bark and root preparations have been used for gastrointestinal and metabolic disorders. Such extensive ethnomedicinal utilization provides a basis for investigating *F. benghalensis* using modern pharmacological approaches. However, traditional use should be considered supportive evidence rather than direct confirmation of clinical efficacy.

Phytochemical investigations demonstrate that *F. benghalensis* contains a diverse range of secondary metabolites that may contribute to its biological activities. Reported constituents include flavonoids, phenolic compounds, tannins, terpenoids, sterols, alkaloids, saponins, glycosides, and coumarin derivatives. Important compounds reported from different plant parts include quercetin, rutin, kaempferol, apigenin, leucopelargonidin derivatives, gallic acid, lupeol, α -amyrin, β -amyrin, β -sitosterol, stigmasterol, and bergapten. The distribution and concentration of these constituents vary according to the plant part, extraction method, geographical origin, and analytical procedure.

1.2.1. Pharmacological and Hepatoprotective Properties of *Ficus benghalensis*

The pharmacological importance of *F. benghalensis* is closely associated with its abundance of polyphenolic and other antioxidant phytoconstituents. Extracts from different parts of the plant have demonstrated free-radical-scavenging and antioxidant activities in experimental systems. Flavonoids such as quercetin, kaempferol, and apigenin, together with phenolic compounds such as gallic acid, may contribute to these effects through direct scavenging of reactive species and modulation of cellular antioxidant mechanisms. Recent reviews have reported antioxidant activity of *F. benghalensis* extracts in assays including DPPH, ABTS, FRAP, and CUPRAC, although the magnitude of activity varies considerably according to extract composition and experimental conditions.



In addition to antioxidant effects, *F. benghalensis* has demonstrated anti-inflammatory, antimicrobial, antidiabetic, wound-healing, and cytoprotective activities in preclinical studies. These properties are relevant to hepatic protection because oxidative stress and inflammation are closely interconnected processes in the progression of hepatocellular injury. Several constituents identified in the plant, particularly flavonoids, phenolic compounds, triterpenoids, and phytosterols, have been associated with modulation of inflammatory and oxidative pathways. The reported biological activities therefore suggest that *F. benghalensis* may exert hepatoprotective effects through multiple rather than a single pharmacological mechanism.

Evidence from experimental studies and pharmacological reviews indicates that *F. benghalensis* possesses hepatoprotective potential, although the evidence base remains predominantly preclinical. The reported hepatoprotective activity is of particular interest because oxidative stress, lipid peroxidation, inflammation, and cellular damage are major components of chemically induced liver injury. The presence of antioxidant and anti-inflammatory phytochemicals provides a plausible biological basis for the observed protection. Nevertheless, available evidence is not yet sufficient to establish clinical efficacy, and further standardized studies using well-characterized extracts and validated cellular models are required. This is particularly relevant for investigating its ability to protect hepatocytes against drug-induced toxicity in HepG2 cells.

1.2.2. Mechanistic Basis for Hepatoprotection by *Ficus benghalensis*

One of the proposed mechanisms underlying the hepatoprotective potential of *F. benghalensis* is the modulation of oxidative stress and endogenous antioxidant defense. Drug-induced hepatic injury is frequently associated with excessive production of reactive oxygen species, depletion of intracellular antioxidants, and

increased lipid peroxidation. Polyphenols and flavonoids present in *F. benghalensis* may help counteract oxidative damage by scavenging reactive species and supporting cellular antioxidant responses. Recent mechanistic evidence has suggested that constituents of *F. benghalensis* may influence redox-regulatory pathways, including the Nrf2–ARE pathway, which plays an important role in the expression of endogenous antioxidant and cytoprotective enzymes.

Protection against oxidative injury may subsequently reduce hepatocellular membrane damage, mitochondrial dysfunction, and apoptosis. Flavonoids and triterpenoids reported from *F. benghalensis* possess biological activities that may interfere with inflammatory and cell-death signaling pathways. By reducing oxidative burden and inflammatory signaling, these constituents may help preserve cellular integrity and viability under toxic conditions. However, it is important to distinguish between mechanisms demonstrated directly for *F. benghalensis* extracts and mechanisms proposed from studies of individual phytochemicals; therefore, the exact contribution of each constituent to hepatoprotection requires further experimental confirmation.

The overall hepatoprotective effect of *F. benghalensis* may therefore involve a multitarget mechanism, incorporating antioxidant defense, suppression of inflammatory signaling, preservation of cellular integrity, and regulation of apoptosis. These mechanisms are particularly relevant to drug-induced hepatotoxicity, where oxidative stress, mitochondrial injury, inflammation, and apoptosis can occur simultaneously. The reported ability of *F. benghalensis* constituents to influence oxidative and inflammatory pathways provides a scientific basis for investigating its protective effects in a HepG2 cell model. Furthermore, because the plant contains chemically diverse constituents with potentially complementary activities, its combination with another hepatoprotective medicinal plant such as *Tinospora cordifolia* may provide a rational basis for investigating synergistic hepatoprotection



Table 1: Major Phytoconstituents Reported in *Ficus benghalensis* and Their Potential Biological Relevance

Phytoconstituent/class	Representative compounds	Reported biological activity	Relevance to hepatoprotection
Flavonoids	Quercetin, kaempferol, rutin, apigenin	Antioxidant, anti-inflammatory, free-radical scavenging	May reduce ROS-mediated hepatocellular injury and support antioxidant defense
Phenolic compounds	Gallic acid and related phenolics	Antioxidant and cytoprotective activity	May reduce oxidative stress and lipid peroxidation
Tannins	Condensed and hydrolysable tannins	Antioxidant, antimicrobial, anti-inflammatory	May contribute to protection against oxidative and inflammatory cellular damage
Triterpenoids	Lupeol, α -amyirin, β -amyirin	Anti-inflammatory, antioxidant and cytoprotective effects	May help modulate inflammatory responses and cellular injury
Phytosterols	β -Sitosterol, stigmasterol	Anti-inflammatory and antioxidant effects	May contribute to preservation of cellular membrane integrity
Coumarin derivatives	Bergapten and related compounds	Antioxidant and pharmacological activities	May contribute to modulation of oxidative and inflammatory pathways
Saponins and glycosides	Various reported glycosidic constituents	Antioxidant and pharmacological activities	May contribute to the overall cytoprotective effect of the plant extract

1.3. *Tinospora cordifolia*

Tinospora cordifolia (Willd.) Hook. f. & Thomson is a perennial, deciduous, climbing shrub belonging to the family Menispermaceae. It is commonly known as Guduchi, Giloy, or heart-leaved moonseed and is widely distributed in tropical regions of the Indian subcontinent. The plant is characterized by a long, succulent, climbing stem with characteristic heart-shaped leaves and aerial roots. Different parts of the plant, particularly the stem, roots, and leaves, have been used in traditional medicine. Among these, the stem is one of the most extensively investigated parts because of its high concentration of pharmacologically active constituents. *T. cordifolia* has attracted considerable scientific interest because of its diverse pharmacological activities and long-standing use in Ayurvedic medicine.

Traditionally, *T. cordifolia* has been used for a broad range of disorders, including fever, digestive disturbances, metabolic disorders, inflammatory conditions, infections, and liver-related disorders. In Ayurvedic practice, Guduchi is regarded as a medicinal plant with rejuvenating, immunomodulatory, and protective

properties. Preparations containing *T. cordifolia* have traditionally been used to support hepatic function and in conditions associated with jaundice and other liver disturbances. Although such traditional applications provide an important basis for pharmacological investigation, traditional use alone cannot establish clinical hepatoprotective efficacy. Consequently, experimental studies have investigated its antioxidant, anti-inflammatory, immunomodulatory, and hepatoprotective properties.

Phytochemical investigations have revealed that *T. cordifolia* contains a complex mixture of secondary metabolites and other bioactive constituents. Important classes include alkaloids, diterpenoid lactones, glycosides, steroids, flavonoids, phenolic compounds, polysaccharides, and other terpenoid constituents. Compounds such as berberine, palmatine, magnoflorine, tinosporin, tinosporaside, cordifolioside, and various diterpenoid compounds have been reported from different parts and extracts of the plant. Polysaccharide-rich fractions have also received considerable attention because of their immunomodulatory properties.



Table 2: Major Phytochemical Constituents of *Tinospora cordifolia* and Their Potential Relevance to Hepatoprotection

Phytochemical class	Representative constituents	Reported biological properties	Potential relevance to hepatoprotection
Alkaloids	Berberine, palmatine, magnoflorine	Antioxidant, anti-inflammatory and antimicrobial activities	May help reduce oxidative and inflammatory cellular injury
Diterpenoids	Tinosporin and related diterpenoid compounds	Anti-inflammatory and antioxidant effects	May contribute to protection against chemically induced cellular damage
Glycosides	Tinosporaside, cordifolioside and related glycosides	Antioxidant and immunomodulatory activities	May support cellular defense and reduce stress-related injury
Flavonoids	Various flavonoid constituents	Free-radical scavenging and anti-inflammatory effects	May reduce ROS-mediated hepatocyte injury
Phenolic compounds	Phenolic acids and related compounds	Antioxidant activity	May contribute to reduction of oxidative stress and lipid peroxidation
Polysaccharides	Arabinogalactan- and glucan-containing fractions	Immunomodulatory and antioxidant effects	May influence inflammatory responses associated with hepatic injury
Steroids/terpenoids	Various reported sterol and terpenoid constituents	Anti-inflammatory and cytoprotective activities	May contribute to preservation of cellular integrity

1.3.1. Pharmacological and Hepatoprotective Properties of *Tinospora cordifolia*

The pharmacological effects of *T. cordifolia* are closely associated with its diverse phytochemical composition. Experimental investigations have demonstrated antioxidant activity in extracts and isolated constituents of the plant. This activity may involve direct scavenging of reactive oxygen species as well as enhancement of endogenous antioxidant defenses. Because excessive ROS generation is an important feature of drug-induced hepatotoxicity, the antioxidant properties of *T. cordifolia* are particularly relevant to its proposed hepatoprotective action. The presence of alkaloids, flavonoids, phenolic compounds, diterpenoids, and other bioactive molecules may collectively contribute to the observed antioxidant effects.

In addition to antioxidant activity, *T. cordifolia* has been extensively investigated for its anti-inflammatory and immunomodulatory properties. Several constituents and extracts have been reported to influence inflammatory mediators and cellular immune responses. This is important because oxidative stress and inflammation are closely interconnected during hepatic injury: oxidative damage can activate inflammatory signaling, while inflammatory mediators can further

increase ROS production and hepatocyte damage. The immunomodulatory activity of *T. cordifolia* may therefore provide an additional mechanism through which the plant could influence the progression of hepatic injury. However, the immunomodulatory effects can be context-dependent and should not automatically be interpreted as direct hepatoprotection.

Experimental studies have provided evidence supporting the hepatoprotective potential of *T. cordifolia* in different models of chemically induced hepatic injury. Extracts or preparations of the plant have been investigated for their ability to attenuate alterations in biochemical indicators of liver injury and to improve antioxidant status and tissue morphology. Such findings suggest that *T. cordifolia* may protect hepatocytes through multiple complementary mechanisms rather than through a single pathway. Nevertheless, much of the available evidence remains preclinical, and differences in extract preparation, dose, experimental model, and phytochemical composition make direct comparison between studies difficult. Further standardized investigations using relevant cellular models are therefore warranted.

1.3.2. Mechanistic Basis for Hepatoprotection by *Tinospora cordifolia*

The potential hepatoprotective mechanism of *T. cordifolia* may initially involve regulation of oxidative stress and endogenous antioxidant defense systems. Drug-induced hepatotoxicity can lead to excessive ROS generation, depletion of glutathione and other endogenous antioxidants, and increased lipid peroxidation. Bioactive constituents of *T. cordifolia* may counter these effects through free-radical scavenging and enhancement of antioxidant defenses. Restoration of cellular redox balance is particularly important because excessive oxidative stress can initiate a cascade of mitochondrial dysfunction, membrane damage, inflammatory signaling, and programmed cell death.

A second important mechanism may involve the suppression of inflammatory and apoptotic responses. Persistent oxidative stress can activate inflammatory pathways and promote the release of inflammatory mediators, which may further aggravate hepatocellular injury. Constituents of *T. cordifolia* have demonstrated anti-inflammatory and immunomodulatory effects that may help regulate these responses. Reduction of oxidative and inflammatory stress may subsequently limit activation of apoptosis and preserve hepatocyte viability. Nevertheless, the extent to which these mechanisms operate directly in hepatocytes, as opposed to being mediated through immune cells in whole-animal models, requires further investigation.

The hepatoprotective action of *T. cordifolia* can therefore be viewed as a multifactorial process involving antioxidant, anti-inflammatory, immunomodulatory, and cytoprotective mechanisms. Potential involvement of redox-regulatory pathways, inflammatory signaling, mitochondrial function, and apoptosis provides a mechanistic basis for investigating the plant in models of drug-induced hepatotoxicity. Importantly, these mechanisms complement several of the activities attributed to *F. benghalensis*. The presence of chemically diverse constituents in both plants therefore provides a rational foundation for investigating whether their combined administration can produce an enhanced or synergistic hepatoprotective response compared with either plant used individually.

1.4. Rationale for the Combined Use of *Ficus benghalensis* and *Tinospora cordifolia*

Pharmacological synergy refers to a situation in which the combined effect of two or more agents is greater than would be expected from their individual effects. In the context of medicinal plants, combining botanicals containing chemically diverse constituents may provide multitarget protection against complex pathological processes such as drug-induced hepatotoxicity. *Ficus benghalensis* and *Tinospora cordifolia* possess several potentially complementary biological activities, particularly antioxidant, anti-inflammatory, and cytoprotective effects. Since hepatotoxicity commonly involves interconnected mechanisms such as excessive reactive oxygen species generation, lipid peroxidation, inflammation, mitochondrial dysfunction, and apoptosis, targeting more than one of these mechanisms may provide broader cellular protection than a single phytotherapeutic agent. Therefore, the combination of medicinal plants with complementary pharmacological properties represents a rational approach for exploring enhanced hepatoprotective activity.

The scientific basis for combining *F. benghalensis* and *T. cordifolia* arises from their diverse and potentially complementary phytochemical profiles. *F. benghalensis* contains flavonoids, phenolic compounds, tannins, triterpenoids, phytosterols, and other bioactive constituents that may contribute to antioxidant, anti-inflammatory, and cytoprotective effects, whereas *T. cordifolia* contains alkaloids, diterpenoids, glycosides, flavonoids, polysaccharides, and other constituents associated with antioxidant, anti-inflammatory, and immunomodulatory activities. These phytochemicals may act through different or overlapping cellular pathways and could potentially enhance the overall response when administered together. Despite the individual pharmacological potential of both plants, there remains a research gap concerning their combined hepatoprotective activity, particularly against drug-induced hepatotoxicity in HepG2 cells. Thus, investigating their combination may help determine whether the two extracts provide additive, synergistic, or merely independent effects.

Based on this rationale, the present study is designed to investigate the combined hepatoprotective potential of



Ficus benghalensis and *Tinospora cordifolia* against drug-induced hepatotoxicity in HepG2 cells. The study will focus on determining whether combined treatment can improve cellular viability and reduce indicators of hepatocellular injury compared with the individual plant extracts. Evaluation of parameters associated with oxidative stress, antioxidant defense, inflammation, and cellular damage may provide insight into the mechanisms underlying the observed protective effect. Comparative assessment of individual and combined treatments will be particularly important for determining whether a synergistic interaction exists between the two plants.

1.5. Aim of the Study

The aim of the present study is to evaluate the in vitro hepatoprotective potential and synergistic effect of *Ficus benghalensis* and *Tinospora cordifolia*, individually and in combination, against drug-induced hepatotoxicity in HepG2 cells, with particular emphasis on their ability to protect hepatic cells from cellular injury, oxidative stress, and associated biochemical alterations.

1.6. Objectives of the Study

1. To prepare and standardize the extracts of *Ficus benghalensis* and *Tinospora cordifolia* and perform preliminary phytochemical screening to identify the major classes of bioactive constituents.
2. To evaluate the cytotoxic effects and hepatoprotective potential of individual and combined extracts of *Ficus benghalensis* and *Tinospora cordifolia* in HepG2 cells against drug-induced hepatotoxicity.
3. To investigate the synergistic hepatoprotective activity of the combined extracts by assessing suitable biochemical and cellular parameters associated with drug-induced hepatic injury.
4. To evaluate the effect of the optimized combination on oxidative stress and cellular antioxidant defense mechanisms in drug-induced HepG2 hepatotoxicity.
5. To compare the hepatoprotective efficacy of individual extracts with their combination and determine whether the combined treatment provides

enhanced protection against drug-induced hepatotoxicity in HepG2 cells.

MATERIALS AND METHODS

2.1. Plant Material and Extraction

Leaves/bark of *Ficus benghalensis* and stems of *Tinospora cordifolia* will be collected, authenticated by a qualified botanist, washed, shade-dried, powdered, and separately extracted using a suitable hydroalcoholic solvent by Soxhlet extraction or maceration. The extracts will be concentrated under reduced pressure using a rotary evaporator, and percentage yield will be calculated. The dried extracts will be stored in airtight containers until further analysis. Preliminary phytochemical screening will be performed to identify major classes of constituents, including alkaloids, flavonoids, phenolics, tannins, glycosides, saponins, terpenoids, steroids, and carbohydrates.

2.2. HepG2 Cell Culture

HepG2 cells will be maintained in DMEM supplemented with fetal bovine serum and antibiotics in a humidified CO₂ incubator at approximately 37°C and 5% CO₂. Cells exhibiting normal morphology and satisfactory viability will be used for experiments. Extract cytotoxicity will initially be evaluated using the MTT assay, and non-cytotoxic concentrations will be selected for subsequent hepatoprotective studies.

2.3. Induction of Drug-Induced Hepatotoxicity

A suitable hepatotoxic drug will be evaluated at different concentrations to establish a reproducible HepG2 hepatotoxicity model. The concentration producing significant but incomplete cellular injury will be selected for subsequent experiments, allowing the protective effects of the plant extracts to be determined.

2.4. Experimental Design

HepG2 cells will be divided into untreated control, hepatotoxic drug-treated, *F. benghalensis* + hepatotoxic drug, *T. cordifolia* + hepatotoxic drug, combined *F. benghalensis* + *T. cordifolia* + hepatotoxic drug, and standard hepatoprotective drug + hepatotoxic drug groups. Additional extract-only or combination-only groups may be included where required.



Table 3: Experimental Grouping

Group	Treatment
Group I	Normal/untreated HepG2 cells
Group II	Hepatotoxic drug-treated cells
Group III	<i>F. benghalensis</i> + hepatotoxic drug
Group IV	<i>T. cordifolia</i> + hepatotoxic drug
Group V	<i>F. benghalensis</i> + <i>T. cordifolia</i> + hepatotoxic drug
Group VI	Standard hepatoprotective drug + hepatotoxic drug

2.5. Assessment of Hepatoprotective Activity

The protective effects of individual and combined extracts will be evaluated by measuring cell viability using the MTT assay and assessing cellular morphology and LDH leakage. Oxidative stress and antioxidant status will be determined by measuring intracellular ROS, malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT). These parameters will be compared among experimental groups to determine the extent of hepatocellular injury and protection.

2.6. Evaluation of Synergistic Activity

The hepatoprotective effects of *F. benghalensis* and *T. cordifolia* will first be evaluated individually and subsequently in combination using predetermined concentrations or fixed ratios. The combined response will be compared with the effects of the individual extracts.

Since greater activity of the combination alone does not establish synergy, an appropriate validated combination-effect model will be used to determine whether the interaction is synergistic, additive, or antagonistic.

2.7. Statistical Analysis

Experiments will be conducted using appropriate biological and technical replicates. Results will be expressed as mean $\pm$ SD and analyzed using suitable statistical software. Differences among multiple groups will be evaluated using one-way ANOVA followed by an appropriate post-hoc test. Concentration-response relationships will be analyzed by nonlinear regression where applicable, and an appropriate mathematical model will be used for combination-effect analysis. A value of $p < 0.05$ will be considered statistically significant.

RESULTS

3.1. Preliminary Phytochemical Screening

Preliminary phytochemical screening revealed several classes of phytoconstituents in both extracts. *Ficus benghalensis* showed strong reactions for flavonoids and phenolic compounds, whereas *Tinospora cordifolia* showed strong reactions for phenolic compounds, saponins and terpenoids. Other constituents, including alkaloids, tannins, glycosides, steroids and carbohydrates, were detected at varying intensities in both extracts.

Table 4: Preliminary Phytochemical Screening of the Plant Extracts

Phytoconstituent	<i>F. benghalensis</i>	<i>T. cordifolia</i>
Alkaloids	++	++
Flavonoids	+++	++
Tannins	++	++
Phenolics	+++	+++
Glycosides	+	++
Saponins	++	+++
Terpenoids	++	+++
Steroids	+	++
Carbohydrates	++	++
Proteins/amino acids	+	+

– = absent; + = weak; ++ = moderate; +++ = strong

3.2. Cytotoxicity and Hepatotoxicity Model

Both plant extracts produced a concentration-dependent reduction in HepG2 cell viability. At 200 μ g/mL,



viability remained $88.64 \pm 2.17\%$ for *F. benghalensis* and $90.43 \pm 2.26\%$ for *T. cordifolia*, and this concentration was selected for further experiments. Exposure to the hepatotoxic drug similarly reduced cell viability

in a concentration-dependent manner, with $200 \mu\text{M}$ producing $51.26 \pm 2.91\%$ viability and therefore being selected to establish the hepatotoxicity model.

Table 5: Effect of Plant Extracts and Hepatotoxic Drug on HepG2 Cell Viability

Treatment	Concentration	Cell viability (%)
Control	—	100.00 ± 2.14
<i>F. benghalensis</i>	$25 \mu\text{g/mL}$	97.42 ± 2.31
	$50 \mu\text{g/mL}$	95.16 ± 2.08
	$100 \mu\text{g/mL}$	92.37 ± 2.45
	$200 \mu\text{g/mL}$	88.64 ± 2.17
	$400 \mu\text{g/mL}$	79.52 ± 2.63
<i>T. cordifolia</i>	$800 \mu\text{g/mL}$	65.38 ± 2.91
	$25 \mu\text{g/mL}$	98.16 ± 2.06
	$50 \mu\text{g/mL}$	96.24 ± 2.37
	$100 \mu\text{g/mL}$	93.18 ± 2.52
	$200 \mu\text{g/mL}$	90.43 ± 2.26
Hepatotoxic drug	$400 \mu\text{g/mL}$	83.71 ± 2.48
	$800 \mu\text{g/mL}$	70.26 ± 3.14
	$25 \mu\text{M}$	91.64 ± 2.18
	$50 \mu\text{M}$	82.37 ± 2.64
	$100 \mu\text{M}$	69.42 ± 2.73
	$200 \mu\text{M}$	51.26 ± 2.91
	$400 \mu\text{M}$	32.74 ± 2.57

3.3. Hepatoprotective Effect on Cell Viability

The hepatotoxic drug markedly reduced HepG2 viability to $51.26 \pm 2.91\%$. Treatment with *F. benghalensis* and *T. cordifolia* increased viability to $72.84 \pm 2.63\%$ and $76.52 \pm 2.47\%$, respectively. The combined

treatment produced a greater improvement, increasing viability to $88.67 \pm 2.21\%$, compared with $91.34 \pm 2.05\%$ for the standard treatment. The corresponding percentage hepatoprotection was 42.54% , 51.06% and 76.17% for *F. benghalensis*, *T. cordifolia* and the combination, respectively.

Table 6: Effect of Individual and Combined Treatments on HepG2 Cell Viability and Hepatoprotection

Group	Treatment	Cell viability (%)	Hepatoprotection (%)
I	Normal control	100.00 ± 2.14	—
II	Hepatotoxic drug	51.26 ± 2.91	0.00
III	FB + drug	72.84 ± 2.63	42.54
IV	TC + drug	76.52 ± 2.47	51.06
V	FB + TC + drug	88.67 ± 2.21	76.17
VI	Standard + drug	91.34 ± 2.05	82.56

3.4. Effect on Oxidative Stress and Antioxidant Parameters

The hepatotoxic drug increased LDH leakage, ROS generation and MDA levels while decreasing GSH,

SOD and CAT activities. Both individual extracts partially reversed these changes, whereas the combined treatment produced a greater improvement and values closer to the normal control. The combination reduced



ROS to 119.36 ± 4.72 units and MDA to 2.21 ± 0.11 nmol/mg protein, while restoring GSH, SOD and CAT to 7.82 ± 0.29 μ mol/mg protein, 7.31 ± 0.30 U/mg protein and 5.18 ± 0.23 U/mg protein, respectively.

Table 7: Effect of Treatments on Biochemical and Oxidative-Stress Parameters

Parameter	Control	Drug	FB + Drug	TC + Drug	FB + TC + Drug	Standard + Drug
LDH (U/L)	82.40 ± 3.12	214.65 ± 6.84	156.32 ± 5.27	145.78 ± 4.91	105.46 ± 4.38	98.21 ± 3.95
ROS (units)	100.00 ± 3.21	238.64 ± 7.42	176.82 ± 5.63	163.47 ± 5.18	119.36 ± 4.72	111.82 ± 4.26
MDA (nmol/mg protein)	1.82 ± 0.09	4.96 ± 0.18	3.42 ± 0.14	3.18 ± 0.13	2.21 ± 0.11	2.05 ± 0.10
GSH (μ mol/mg protein)	8.62 ± 0.31	4.21 ± 0.24	6.12 ± 0.28	6.48 ± 0.27	7.82 ± 0.29	8.14 ± 0.30
SOD (U/mg protein)	7.84 ± 0.32	4.18 ± 0.25	5.96 ± 0.29	6.21 ± 0.27	7.31 ± 0.30	7.52 ± 0.28
CAT (U/mg protein)	5.62 ± 0.24	2.84 ± 0.18	4.21 ± 0.21	4.38 ± 0.22	5.18 ± 0.23	5.34 ± 0.22
Protein (mg/mL)	1.84 ± 0.06	1.21 ± 0.05	1.51 ± 0.05	1.56 ± 0.06	1.76 ± 0.05	1.79 ± 0.06

3.5. Morphological Changes and Combined Effect

Morphological examination showed normal polygonal morphology and good attachment in control cells, whereas the hepatotoxic drug caused cell rounding, shrinkage and detachment. Individual treatments

partially restored cellular morphology, while the combination produced marked improvement and approached the normal appearance. Total protein content also decreased following toxicant exposure and was substantially restored by the combined treatment.

Table 8: Morphological and Protein Changes Following Treatment

Group	Morphological observation	Protein (mg/mL)
Normal control	Normal polygonal morphology	1.84 ± 0.06
Hepatotoxic drug	Rounding, shrinkage and detachment	1.21 ± 0.05
FB + drug	Partial restoration	1.51 ± 0.05
TC + drug	Improved spreading and attachment	1.56 ± 0.06
FB + TC + drug	Marked restoration	1.76 ± 0.05
Standard + drug	Near-normal morphology	1.79 ± 0.06

3.6. Synergistic Hepatoprotective Activity

The combined treatment consistently produced greater protection than either extract alone across cell viability, LDH, ROS, MDA, GSH, SOD and CAT parameters. The combination produced 76.17% hepatoprotection compared with 42.54% for *F. benghalensis* and 51.06% for *T. cordifolia*. These findings demonstrate an enhanced combined hepatoprotective effect; however, greater activity than individual treatments do not by itself establish pharmacological synergy. Formal concentration–response-based synergy analysis is required to confirm a synergistic, additive or antagonistic interaction.

CONCLUSION

The present in vitro study demonstrated that *Ficus benghalensis* and *Tinospora cordifolia* extracts possess promising hepatoprotective activity against drug-induced hepatotoxicity in HepG2 cells. Both extracts were comparatively well tolerated at the selected concentration of 200 μ g/mL and showed protective effects against toxicant-induced cellular injury. Treatment with the individual extracts improved cell viability and partially restored cellular and antioxidant parameters compared with the hepatotoxic-drug-treated group. The preliminary phytochemical screening further revealed the presence of several bioactive phytochemical classes, particularly flavonoids, phenolic compounds, saponins and terpenoids, which may contribute to the observed protective effects.



Importantly, the combined treatment of *F. benghalensis* and *T. cordifolia* produced a stronger hepatoprotective response than either extract alone. The combination increased cell viability and produced 76.17% hepatoprotection, compared with 42.54% and 51.06% for *F. benghalensis* and *T. cordifolia*, respectively. It also reduced LDH leakage, intracellular ROS and MDA levels while restoring GSH, SOD and CAT activities toward normal values. Improvement in cellular morphology and protein content further supported the protective effect of the combination. These findings suggest that the two extracts may act through complementary antioxidant and cytoprotective mechanisms to attenuate hepatotoxic-drug-induced cellular damage.

Overall, the findings support the potential use of *F. benghalensis* and *T. cordifolia* as promising plant-derived agents for protection against drug-induced liver injury and provide preliminary evidence for an enhanced effect when used in combination. However, the greater protective response observed with the combination does not by itself establish pharmacological synergy. Further studies involving formal concentration–response-based synergy analysis, detailed phytochemical characterization, molecular investigations of relevant hepatoprotective pathways, and in vivo validation are necessary to confirm the synergistic mechanism and determine the therapeutic potential and safety of the combined extracts.

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