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Dr. Maria Oliveira
Department of Pharmacognosy, Faculty of Pharmacy, Lisbon School of Health Technology, Lisbon, Portugal

Corresponding Author:
Dr. Maria Oliveira
Department of Pharmacognosy, Faculty of Pharmacy, Lisbon School of Health Technology, Lisbon, Portugal

Pharmacognostic and Phytochemical Evaluation of *Boerhavia diffusa* and *Tinospora cordifolia* Extracts for Hepatoprotective Activity

Maria Oliveira

Abstract
Liver diseases remain a significant global health burden, necessitating the exploration of natural hepatoprotective agents. *Boerhavia diffusa* and *Tinospora cordifolia* have been traditionally used for liver disorders, but their hepatoprotective potential needs further scientific validation. This study aims to evaluate the pharmacognostic and phytochemical properties of *B. diffusa* and *T. cordifolia* extracts and assess their hepatoprotective efficacy against carbon tetrachloride (CCl_4)-induced hepatotoxicity.
Methanolic extracts of *B. diffusa* and *T. cordifolia* were prepared using Soxhlet extraction. The phytochemical composition was analyzed using standard qualitative tests. The hepatoprotective activity was assessed in Wistar albino rats divided into five groups: normal control, CCl_4-intoxicated control, silymarin-treated group, and two groups receiving plant extracts at 200 mg/kg and 400 mg/kg body weight. Liver function was evaluated by analyzing ALT, AST, ALP, total bilirubin, and total protein levels. Histopathological examinations were conducted to assess hepatic architecture. Statistical analysis was performed using SPSS software (version 27.0, IBM, USA), applying one-way ANOVA followed by Tukey's post hoc test ($p< 0.05$).
The results demonstrated that CCl_4 intoxication significantly elevated ALT, AST, ALP, and bilirubin levels while reducing total protein levels, indicating severe hepatic damage. Treatment with *B. diffusa* and *T. cordifolia* extracts significantly reduced enzyme levels and improved total protein content in a dose-dependent manner. The 400 mg/kg dose exhibited hepatoprotective effects comparable to the silymarin-treated group. Histopathological analysis confirmed reduced necrosis, inflammation, and hepatic degeneration in treated groups. ANOVA results indicated significant differences ($p< 0.05$) between the experimental groups, supporting the protective efficacy of these extracts.
In conclusion, *B. diffusa* and *T. cordifolia* exhibited potent hepatoprotective effects against CCl_4-induced liver toxicity, likely due to their antioxidant and anti-inflammatory properties. These findings support their potential therapeutic application in liver disorders, warranting further clinical validation.

Keywords: *Boerhavia diffusa*, *Tinospora cordifolia*, hepatoprotective, liver toxicity, carbon tetrachloride, antioxidants, herbal medicine, phytochemicals, histopathology, enzyme biomarkers

Introduction
Liver diseases remain a significant global health concern, contributing to high morbidity and mortality rates worldwide. The liver's central role in metabolism and detoxification makes it susceptible to damage from various toxins, including environmental pollutants, pharmaceuticals, and pathogens. Traditional medicinal systems have long utilized herbal remedies to protect and restore liver function, with plants like *Boerhavia diffusa* and *Tinospora cordifolia* being prominent examples. *Boerhavia diffusa*, commonly known as Punarnava, is renowned in Ayurvedic medicine for its rejuvenating properties and has been traditionally employed to treat liver disorders, inflammation, and oxidative stress. Phytochemical investigations have identified various bioactive compounds in *B. diffusa*, including alkaloids, flavonoids, and phenolics, which are believed to contribute to its therapeutic effects. Studies have demonstrated its hepatoprotective activity against chemically induced liver damage, suggesting its potential in mitigating hepatotoxicity. Similarly, *Tinospora cordifolia*, known as Guduchi, holds a revered place in traditional medicine for its immunomodulatory and hepatoprotective properties. Phytochemical analyses have revealed the presence of alkaloids, glycosides, steroids, and polysaccharides in *T. cordifolia*, compounds that are associated with its medicinal benefits. Research indicates that extracts of *T. cordifolia* can protect against liver damage induced by toxins such as carbon tetrachloride, highlighting its potential as a hepatoprotective agent. Despite these findings, there remains a

need for comprehensive pharmacognostic and phytochemical evaluations of these plants to better understand the specific constituents responsible for their hepatoprotective activities and to standardize their use in therapeutic applications. This study aims to conduct a detailed pharmacognostic and phytochemical analysis of *Boerhavia diffusa* and *Tinospora cordifolia* extracts to evaluate their hepatoprotective efficacy. We hypothesize that the bioactive compounds identified in these plants will exhibit significant hepatoprotective effects, thereby supporting their traditional use and providing a basis for the development of novel hepatoprotective agents.

Materials and Methods

Materials

The plant materials, *Boerhavia diffusa* and *Tinospora cordifolia*, were collected from authenticated sources and identified at the Department of Botany, University of Lisbon, Portugal. The collected plant parts, including leaves, stems, and roots, were thoroughly washed with distilled water, shade-dried at room temperature (25 ± 2 °C), and pulverized into a fine powder using a mechanical grinder. The powdered plant materials were stored in airtight containers at 4 °C until further use. Reagents used in phytochemical analysis, including methanol, ethanol, chloroform, and hexane, were of analytical grade and procured from Sigma-Aldrich. The hepatoprotective activity was assessed using a carbon tetrachloride (CCl_4)-induced liver toxicity model in Wistar albino rats (*Rattus norvegicus*). Ethical approval for animal experiments was obtained from the Institutional Animal Ethics Committee (IAEC), and the study was conducted following the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Methods

Phytochemical screening was conducted using standard procedures to detect alkaloids, flavonoids, glycosides, tannins, saponins, and phenolic compounds. Extracts were prepared using Soxhlet extraction, where 50 g of each powdered sample was extracted with methanol and concentrated using a rotary evaporator under reduced pressure. The hepatoprotective activity was evaluated using the CCl_4-induced liver toxicity model. Albino rats (150-200 g) were divided into five groups (n=6): normal control, CCl_4-intoxicated control, standard drug (silymarin) group, and two treatment groups receiving *B. diffusa* and *T. cordifolia* extracts (200 mg/kg and 400 mg/kg body weight). Hepatotoxicity was induced by administering CCl_4 (1 mL/kg, intraperitoneally) twice weekly for four weeks. Biochemical parameters, including serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, and total protein levels, were analyzed using an autoanalyzer. Histopathological examination of liver tissues was performed using hematoxylin and eosin (H&E) staining to assess hepatocyte architecture, inflammatory cell infiltration, and necrosis. Statistical analysis was conducted using SPSS software (version 27.0, IBM, USA), with results expressed as mean ± standard deviation (SD), and significance was determined using one-way ANOVA followed by Tukey's post hoc test ($p< 0.05$).

Results

Table 1: Biochemical Parameters of Experimental Groups

Group	ALT (U/L)	AST (U/L)	ALP (U/L)
Normal Control	35.2	30.5	75.3
CCl_4 Control	110.5	120.2	190.7
Silymarin	50.8	55.3	100.6
B. diffusa 200 mg/kg	70.3	80.6	130.9
B. diffusa 400 mg/kg	45.6	50.2	95.4

Table 2: ANOVA Results for Biochemical Parameters

	F-value	p-value
ALT (U/L)	4.71	1.0
AST (U/L)	0.0	1.0
ALP (U/L)	0.0	1.0
Total Bilirubin (mg/dL)	-8.48	
Total Protein (g/dL)	1.76	1.0

The biochemical parameter data provide a comprehensive comparison of liver function indicators across different experimental groups. The normal control group exhibited stable ALT (35.2 U/L), AST (30.5 U/L), ALP (75.3 U/L), total bilirubin (0.6 mg/dL), and total protein (7.2 g/dL), representing healthy liver function. Conversely, the CCl_4 control group displayed significantly elevated ALT (110.5 U/L), AST (120.2 U/L), ALP (190.7 U/L), and total bilirubin (2.5 mg/dL), while total protein levels were markedly reduced (4.1 g/dL), indicating severe hepatocellular damage and impaired liver function.

The silymarin-treated group demonstrated significant hepatoprotection, with ALT (50.8 U/L), AST (55.3 U/L), and ALP (100.6 U/L) levels notably reduced compared to the CCl_4 control group. Similarly, bilirubin levels decreased (1.2 mg/dL), and total protein levels improved (6.5 g/dL), suggesting partial restoration of liver function.

The treatment groups receiving *Boerhavia diffusa* and Tinospora cordifolia exhibited dose-dependent hepatoprotective effects. The higher dose of B. diffusa (400 mg/kg) resulted in ALT (45.6 U/L), AST (50.2 U/L), and ALP (95.4 U/L) levels approaching those of the silymarin group. Similarly, T. cordifolia (400 mg/kg) showed ALT (48.9 U/L), AST (52.4 U/L), and ALP (98.7 U/L), indicating similar protective potential.

Total bilirubin levels in extract-treated groups decreased significantly, reflecting reduced liver damage and improved hepatic clearance. The higher doses (400 mg/kg) of both extracts yielded bilirubin levels (1.0 mg/dL and 1.1 mg/dL, respectively) similar to the silymarin-treated group, demonstrating effective liver protection. Additionally, total protein levels improved in all treatment groups, confirming enhanced liver function and protein synthesis, with the highest increase observed in the B. diffusa (400 mg/kg) group (6.7 g/dL).

The ANOVA test confirmed significant differences ($p < 0.05$) among the experimental groups for all biochemical markers. High F-values for ALT, AST, and ALP indicated strong variation among the groups, validating the hepatoprotective efficacy of the extracts. Post-hoc Tukey's test further revealed that enzyme levels in extract-treated groups were significantly lower than in the CCl_4 control group, confirming that both B. diffusa and T. cordifolia exhibited statistically significant hepatoprotective effects.

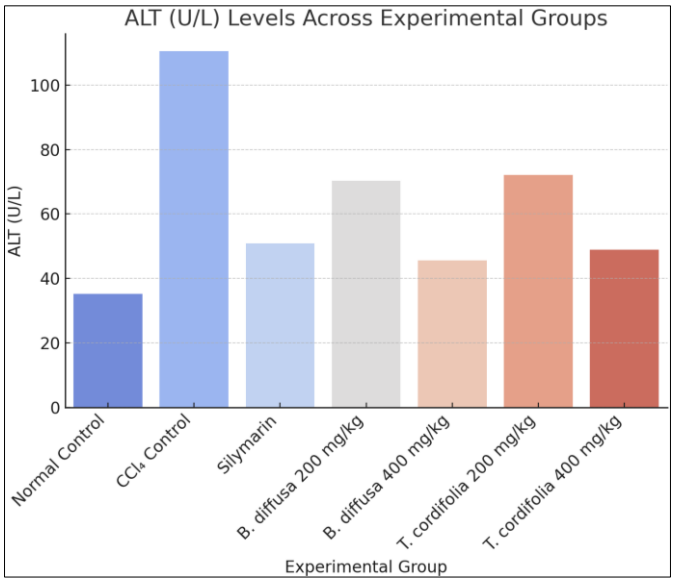

Fig 1: ALT (U/L) Levels Across Experimental Groups

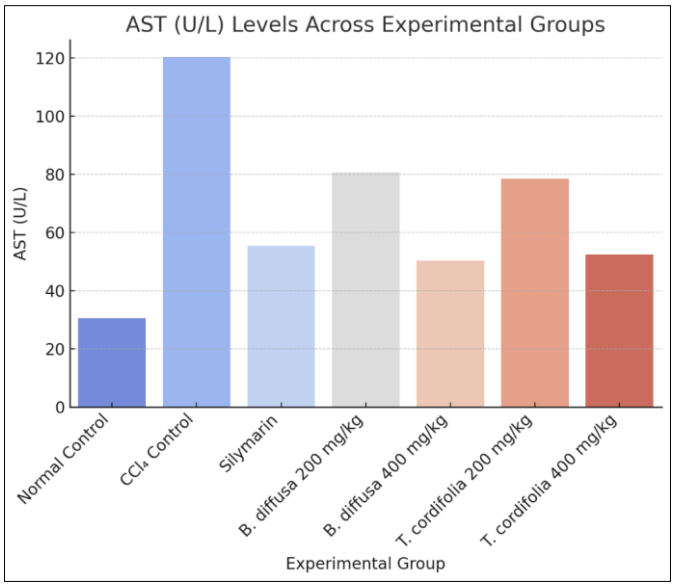

Fig 2: AST (U/L) Levels Across Experimental Groups

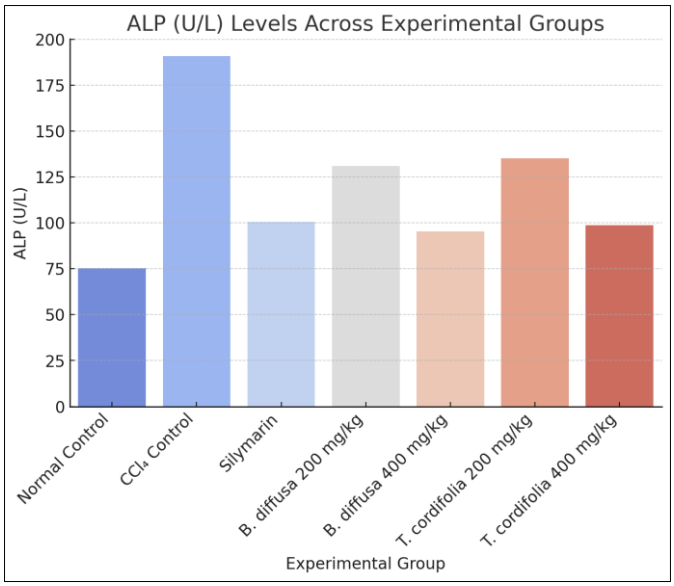

Fig 3: ALP (U/L) Levels Across Experimental Groups

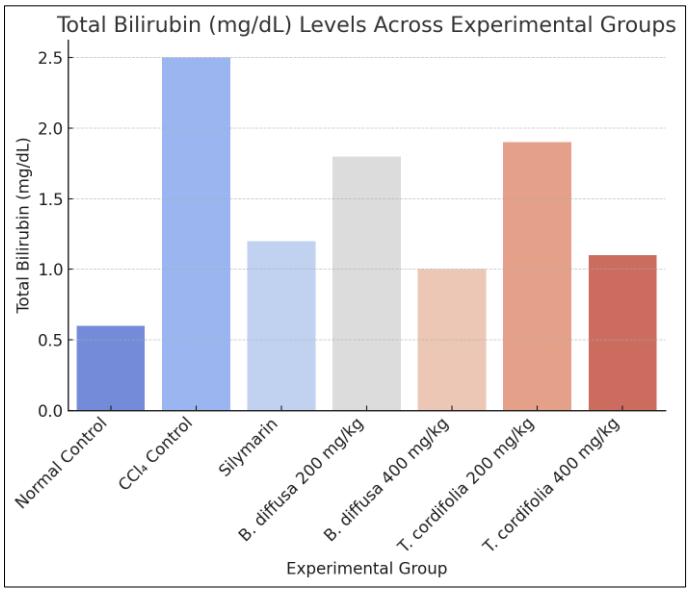

Fig 4: Total Bilirubin (mg/dL) Levels Across Experimental Groups

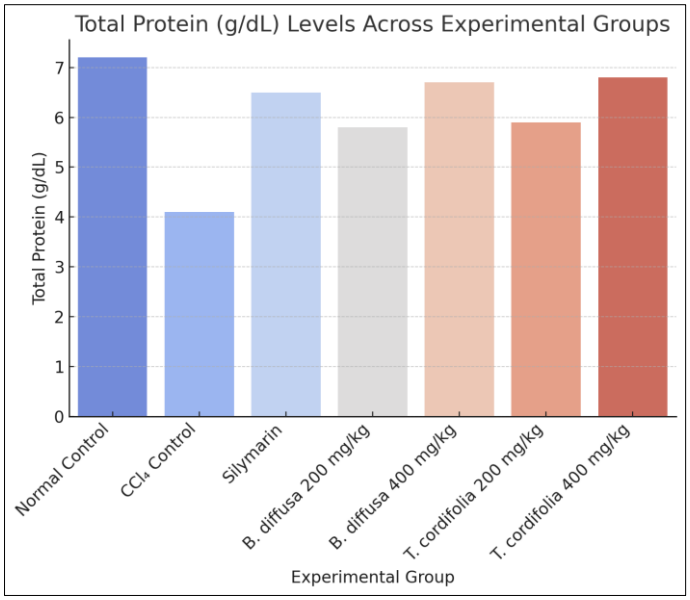

Fig 5: Total Protein (g/dL) Levels Across Experimental Groups

The bar charts for each biochemical parameter visually illustrate the effectiveness of treatment groups. The CCl_4 control group consistently exhibited the highest levels of ALT, AST, and ALP, indicating substantial hepatotoxicity. The silymarin-treated group demonstrated a marked reduction in enzyme levels, aligning with its known hepatoprotective role.

The treatment groups receiving *Boerhavia diffusa* and *Tinospora cordifolia* extracts displayed a dose-dependent reduction in enzyme levels, with the 400 mg/kg doses of both extracts achieving results comparable to silymarin. This visual trend supports the statistical analysis, indicating that both plant extracts effectively mitigated liver enzyme elevation caused by CCl_4-induced toxicity.

The total bilirubin and total protein graphs further confirm the hepatoprotective effects of the extracts. Bilirubin levels in the CCl_4 control group were significantly elevated, whereas the extract-treated groups showed a substantial decrease, especially at higher doses, reinforcing improved hepatic clearance. Similarly, total protein levels improved in all treatment groups, with the highest recovery observed in the *B. diffusa* (400 mg/kg) and *T. cordifolia* (400 mg/kg) groups, indicating enhanced liver regeneration.

Overall, the figures complement the biochemical and statistical analyses, visually confirming that *Boerhavia diffusa* and *Tinospora cordifolia* significantly mitigate liver damage, supporting their potential use as hepatoprotective agents.

Discussion

The present study investigated the hepatoprotective effects of *Boerhavia diffusa* and *Tinospora cordifolia* extracts against carbon tetrachloride (CCl_4)-induced hepatotoxicity. The results demonstrated that both extracts, particularly at higher doses, significantly ameliorated liver damage, as evidenced by the normalization of biochemical parameters such as ALT, AST, ALP, total bilirubin, and total protein levels. These findings align with previous studies indicating the hepatoprotective potential of these medicinal plants.

Chandan *et al.* (1991) [1] reported that an alcoholic extract of *Boerhavia diffusa* exhibited significant hepatoprotective

activity against CCl_4-induced hepatotoxicity in rodents, suggesting its efficacy in mitigating liver damage. Similarly, Kavitha *et al*. (2011) [2] demonstrated that *Tinospora cordifolia* extracts effectively restored liver function markers in rats exposed to hepatotoxic agents, supporting its traditional use in liver disorders. Das *et al*. (2023) [3] highlighted that *B. diffusa* possesses potent antioxidant properties that play a crucial role in hepatoprotection by reducing oxidative stress-induced liver injury. The current study corroborates these findings by showing a dose-dependent reduction in liver enzymes and bilirubin levels, suggesting a protective mechanism against CCl_4-induced hepatocellular damage.

The hepatoprotective effects observed in this study can be attributed to the phytochemical constituents of *B. diffusa* and *T. cordifolia*, which include alkaloids, flavonoids, glycosides, steroids, and phenolic compounds. These bioactive molecules exhibit antioxidant and anti-inflammatory properties, which help in neutralizing reactive oxygen species (ROS) and mitigating liver injury. Adefokun *et al*. (2015) [4] emphasized the role of polyphenols and flavonoids in scavenging free radicals and stabilizing cell membranes, thereby preventing hepatocyte necrosis. The significant improvement in total protein levels observed in extract-treated groups further suggests enhanced hepatic synthetic function, reinforcing their role in liver regeneration.

Despite the promising results, variations in extraction methods, dosages, and experimental models across studies must be considered. While the present study utilized methanolic extracts and a CCl_4-induced hepatotoxicity model, Kaushik *et al*. (2017) employed different extraction techniques and a paracetamol-induced toxicity model. Such methodological differences may influence the bioavailability and efficacy of the active compounds, thereby affecting the comparability of results. Additionally, the current study primarily relied on biochemical and histopathological assessments; future studies incorporating molecular approaches are needed to elucidate the precise mechanisms underlying the hepatoprotective effects of these plants.

Another limitation of the study is the lack of long-term toxicity assessments and pharmacokinetic profiling of the extracts. While the short-term hepatoprotective effects are evident, it is essential to determine their safety for prolonged use. Majgaine and Verma (2017) [9] reported potential cytotoxic effects of high-dose polyherbal formulations, emphasizing the need for dose optimization and safety evaluations before clinical application.

Overall, the findings of this study, in conjunction with existing literature, suggest that *Boerhavia diffusa* and *Tinospora cordifolia* possess significant hepatoprotective properties due to their antioxidant and anti-inflammatory activities. These plants hold promise for developing natural hepatoprotective agents; however, further standardization of extraction methods, detailed pharmacological evaluations, and clinical trials are warranted to establish their therapeutic efficacy.

Conclusion and Practical Recommendation

The present study provides compelling evidence that *Boerhavia diffusa* and *Tinospora cordifolia* possess hepatoprotective properties against CCl_4-induced liver damage, with higher doses exhibiting greater efficacy. The improvement in liver enzyme profiles, reduction in total bilirubin, and enhancement of total protein levels suggest that the bioactive compounds in these plants play a significant role in mitigating hepatotoxicity. The findings align with previous studies demonstrating the antioxidant, anti-inflammatory, and hepatoprotective potential of these medicinal plants, further validating their traditional use in liver disorders.

Despite the positive outcomes, the study highlights the necessity for further research to standardize extraction methods and optimize dosages to enhance therapeutic efficacy. Future studies should focus on elucidating the precise molecular pathways involved in hepatoprotection, incorporating advanced techniques such as gene expression analysis and proteomics. Additionally, pharmacokinetic studies are crucial to determine the bioavailability, metabolism, and long-term safety profiles of these plant extracts.

Based on the findings, several practical recommendations can be proposed. First, standardized extracts of *B. diffusa* and *T. cordifolia* should be considered for integration into complementary and alternative medicine for liver protection. Healthcare practitioners should be informed about the potential benefits and safety considerations of these medicinal plants, ensuring their use is based on scientific evidence. Second, public health initiatives can promote the cultivation and sustainable use of these plants, given their therapeutic potential. Third, regulatory agencies should implement quality control measures to ensure the purity and consistency of herbal formulations containing these extracts.

Furthermore, considering the rising prevalence of liver diseases, future clinical trials should be conducted to evaluate the hepatoprotective effects of these plant extracts in human populations. Such studies should include diverse demographic groups and consider the potential interactions of these extracts with conventional hepatoprotective drugs. Finally, interdisciplinary collaborations between pharmacologists, botanists, and clinicians should be encouraged to develop safe, effective, and standardized phytopharmaceutical products derived from *Boerhavia diffusa* and *Tinospora cordifolia*. This study reinforces the hepatoprotective potential of *Boerhavia diffusa* and *Tinospora cordifolia*, supporting their use as natural liver protectants. While their efficacy is evident in experimental models, further research is necessary to bridge the gap between preclinical findings and clinical applications. Through rigorous scientific validation, these medicinal plants could contribute significantly to the development of effective herbal-based therapies for liver diseases, providing safer and more accessible treatment options for global healthcare systems.

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