Research Article

In vivo Hepatoprotective activity of Hydroalcoholic extract of Bauhinia variegata, Ficus Carica and Solanum nigrum extracts

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Abstract

The present study was undertaken to evaluate the hepatoprotective potential of hydroalcoholic extracts of Bauhinia variegata, Ficus carica and Solanum nigrum, individually and in combination, against carbon tetrachloride (CCl_4)-induced hepatotoxicity in experimental rats. The hepatoprotective activity was assessed by monitoring changes in body weight, serum biochemical parameters, hepatic oxidative stress markers and inflammatory cytokines. Animals were treated with individual plant extracts and their combined formulation at doses of 100 and 200 mg/kg, with silymarin (10 mg/kg) used as the standard reference treatment. Serum biochemical parameters included alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin, albumin and total protein. Hepatic oxidative stress was evaluated by estimating malondialdehyde (MDA), superoxide dismutase (SOD), reduced glutathione (GSH) and catalase (CAT), while inflammatory responses were assessed through TNF-α, IL-1β and IL-6 levels. The CCl_4 control group showed increased ALT, AST, ALP, bilirubin, MDA and inflammatory cytokine levels, accompanied by reduced body weight, SOD, GSH, CAT and total protein levels compared with the normal control group. Treatment with individual plant extracts improved several of these parameters. Among the evaluated treatments, the combined extract at 200 mg/kg demonstrated comparatively better results, with reductions in liver enzyme levels, lipid peroxidation and inflammatory cytokines, along with improvements in antioxidant defence parameters and total protein levels. These findings suggest that the combined hydroalcoholic extracts of the selected medicinal plants possess promising hepatoprotective potential, possibly through antioxidant and anti-inflammatory mechanisms. Further histopathological and mechanistic investigations are required to establish their efficacy and underlying mechanisms.

Keywords: Hepatoprotective activity, Bauhinia variegata, Ficus carica, Solanum nigrum, Carbon tetrachloride, Inflammatory cytokines, Hydroalcoholic extract.

1. Introduction

The liver is one of the most important organs of the human body and plays a vital role in maintaining metabolic homeostasis. It is involved in several physiological processes, including carbohydrate, protein and lipid metabolism, detoxification of harmful substances, synthesis of plasma proteins, bile production and regulation of various biochemical pathways (Rui, 2014; Reshetnyak, 2013). Due to its continuous exposure to drugs, chemicals, environmental toxins and metabolic waste products, the liver is particularly susceptible to toxic injury (Gu and Manautou, 2012). Hepatotoxicity refers to liver damage caused by chemical substances, drugs or other toxic agents and may result in cellular degeneration, inflammation, oxidative stress and impairment of normal hepatic functions (Pandit et al., 2012).

Oxidative stress is considered one of the major mechanisms involved in the development and progression of liver injury. It occurs when the production of reactive oxygen species (ROS) exceeds the antioxidant defence capacity of the body (Cichoż-Lach and Michalak, 2014). Excessive ROS generation can damage cellular membranes, proteins and nucleic acids through oxidative reactions. Lipid peroxidation is an important consequence of oxidative stress and is commonly assessed by measuring malondialdehyde (MDA). The antioxidant defence system includes enzymes such as superoxide dismutase (SOD) and catalase (CAT), along with non-enzymatic antioxidants such as reduced glutathione (GSH). Alterations in these antioxidant parameters are useful indicators of oxidative damage and hepatic dysfunction (Jomova et al., 2023).

Carbon tetrachloride (CCl_4) is a widely used experimental hepatotoxic agent for investigating liver injury and evaluating potential hepatoprotective substances (Mahmoodzadeh et al., 2017). Its administration results in metabolic activation

by hepatic cytochrome P450 enzymes, particularly CYP2E1, leading to the formation of highly reactive trichloromethyl (CCl_3•) and trichloromethyl peroxy (CCl_3OO•) radicals (Fareed et al., 2024). These free radicals initiate lipid peroxidation, disrupt cellular membrane integrity and cause hepatocellular damage. CCl_4-induced liver injury is commonly associated with elevated serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin, along with alterations in albumin and total protein levels. Therefore, these biochemical parameters are frequently used to assess the extent of hepatic injury and the protective effects of test substances (Saha et al., 2024).

Inflammation also plays an important role in the progression of toxic liver injury. The production of pro-inflammatory cytokines, including tumour necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β) and interleukin-6 (IL-6), contributes to inflammatory responses and may aggravate hepatocellular damage (Lacour et al., 2005). Increased cytokine production, together with oxidative stress and lipid peroxidation, can accelerate tissue injury. Consequently, the assessment of inflammatory cytokines, along with liver function and antioxidant parameters, provides a broader understanding of the mechanisms involved in hepatotoxicity and hepatoprotection (Li et al., 2016).

Natural products and medicinal plants have attracted considerable scientific attention as potential sources of hepatoprotective agents. They contain several bioactive phytoconstituents, including flavonoids, phenolic compounds, tannins, terpenoids and saponins, which may contribute to antioxidant and anti-inflammatory activities. The investigation of medicinal plants is therefore important for identifying naturally occurring compounds that may help protect hepatic tissue against toxic injury (Adewusi and Afolayan, 2010).

Bauhinia variegata, commonly known as Kachnar, belongs to the family Fabaceae. It has a long history of traditional medicinal use and contains various phytoconstituents, including flavonoids, tannins, glycosides and phenolic compounds. These constituents have been investigated for their potential antioxidant, anti-inflammatory and other pharmacological activities (Sharma et al., 2021). The presence of these phytochemicals makes *Bauhinia variegata* a promising candidate for further investigation of its possible protective effects against oxidative and inflammatory tissue damage.

Ficus carica, commonly known as fig, belongs to the family Moraceae and is widely recognized for its nutritional and medicinal importance. Different parts of the plant, particularly the fruits and leaves, contain phenolic compounds, flavonoids, tannins and other biologically active constituents. These phytochemicals have been associated with antioxidant, antimicrobial and anti-inflammatory properties (Mawa et al., 2013). The presence of phenolic and flavonoid compounds provides a scientific basis for investigating the potential hepatoprotective properties of *Ficus carica*.

Solanum nigrum, commonly known as black nightshade, belongs to the family Solanaceae and is traditionally used in different systems of medicine. The plant contains several secondary metabolites, including flavonoids, phenolic compounds, saponins, tannins and steroidal constituents. Previous investigations have reported various biological activities, including antioxidant, anti-inflammatory and hepatoprotective properties (Gębarowska et al., 2022). These reported properties support further evaluation of *Solanum nigrum* as a potential source of hepatoprotective phytoconstituents.

The combination of medicinal plant extracts may provide a broader range of phytochemical constituents than individual extracts. Different phytoconstituents may contribute to antioxidant and anti-inflammatory effects through different biological pathways. However, the effectiveness of a combined extract must be established through systematic experimental evaluation rather than assumed from the individual properties of its constituent plants (Nunes et al., 2020).

Considering the medicinal importance and reported phytochemical composition of these plants, the present study was designed to evaluate the hepatoprotective potential of hydroalcoholic extracts of *Bauhinia variegata*, *Ficus carica* and *Solanum nigrum*, individually and in combination, against CCl_4-induced hepatotoxicity in experimental rats. The study involved the assessment of body-weight changes and serum biochemical parameters, including ALT, AST, ALP, bilirubin, albumin and total protein. Furthermore, hepatic oxidative stress was evaluated by estimating MDA, SOD, GSH and CAT, while inflammatory responses were investigated through the measurement of TNF-α, IL-1β and IL-6. The study aimed

to compare the effects of individual and combined plant extracts and explore their possible hepatoprotective potential through antioxidant and anti-inflammatory mechanisms.

2. Material and Methods

2.1 Material

The present study utilized hydroalcoholic extracts of *Bauhinia variegata*, *Ficus carica* and *Solanum nigrum* for evaluating their hepatoprotective activity. Carbon tetrachloride (CCl_4) was used to induce hepatotoxicity, while silymarin was used as the standard drug. Ethanol, methanol, distilled water and other analytical-grade chemicals and reagents were procured from Merck Life Science Pvt. Ltd., Mumbai, India; Loba Chemie Pvt. Ltd., Mumbai, India; and HiMedia Laboratories Pvt. Ltd., Mumbai, India. All reagents were of analytical grade and used without further purification.

2.2 Procurement of plant materials

The leaves of *Bauhinia variegata*, *Ficus carica*, and *Solanum nigrum* were collected in the month March 2024 from a ruler area of Bhopal. After the collection of plants, they have been processed for cleaning to prevent the deterioration of the phytochemicals present in the plant.

2.3 Extraction procedure

Following procedure was adopted for the preparation of extracts from the shade dried and powdered herbs (Khandelwal, 2005; Kokate, 1994).

2.4 Defatting of Plant Materials

Extraction of 50g shade-dried leaves of the *Bauhinia Variegata, Ficus Carica, Solanum Nigrum* with Petroleum ether by the maceration method respectively. The extraction was continued till the defatting of the materials had taken place.

2.5 Extraction with hydroalcoholic solvent by maceration method

Defatted plant material was extracted using hydroalcoholic solvent (ethanol: water 80:20v/v). Maceration method was used to extract plant materials. The resulting solution was filtered with whatman filter paper no.1 and kept for solvent evaporation to yield dry concentrated extract. The dried concentrated crude extract was weighed for the extractive yield determination and transferred into glass vials (6 ×2 cm) and stored in a refrigerator (4°C), till used for the analysis.

2.6 Animals

The present study used male Wistar rats with a body weight of 180±20 g. They were acclimatized and kept under laboratory conditions (constant temperature 24±5 °C relative humidity 50 to 60 %) with 12 h light and dark cycles. All animals of the study were given free feeding and drinking water during all experimental period with standard laboratory pellet diet. The experiments and procedures carried out in the study were performed in in accordance with the recommended guidelines of Organisation for Economic Co-operation and Development (OECD) (ICH S2A, ICH S2B OECD 471 and OECD 452) and approval received from Institutional Animal Ethics Committee.

2.7 Acute toxicity study

The study of oral toxicity was done on a combined extract of *Bauhinia variegata, Ficus carica* and *Solanum nigrum* was performed in male and female albino rats up to a maximum dose limit of 2000 mg/kg as per OECD Guideline 423. The animals were observed for any sign of toxicity and mortality level for a period of 14 days after a single oral administration. The safety and tolerability of the combined extract were assessed in the present study. We assessed for variations in body weight and behaviour, reaction to external stimuli, and the condition of skin and fur, eyes and mucous membranes (Bedi and Krishan, 2020).

2.8 Experimental design and treatment protocol:

Rats were allocated to 11 groups randomly and evenly (n=6) in each group. For duration of four weeks, treatments were given orally once every day (Vuda et al., 2012).

- **Group I:** Normal control; 0.9% saline solution was administered orally.
- **Group II:** CCl_4 0.7 ml/kg, given orally (Bagali *et al.,* 2020)

- **Group -III:** Rats were administered CCl_4 +silymarin 10 mg/kg.
- **Group –IV:** Rats were administered CCl_4 + Extract of *Bauhinia variegata*100 mg/kg
- **Group –V:** Rats were administered CCl_4 + Extract of *Bauhinia variegata*200 mg/kg
- **Group –VI:** Rats were administered CCl_4 + Extract of *Ficus carica* 100 mg/kg
- **Group –VII:** Rats were administered CCl_4 + Extract of *Ficus carica* 200 mg/kg
- **Group –VIII:** Rats were administered CCl_4 + Extract of *Solanum nigrum*100 mg/kg
- **Group –IX:** Rats were administered CCl_4 + Extract of *Solanum nigrum* 200 mg/kg
- **Group –X:** Rats were administered $CCl_4$and the combined extract of *Bauhinia variegata, Ficus carica* and *Solanum nigrum* 100 mg/kg
- **Group –XI:** Rats were administered CCl_4 and the combined extract of *Bauhinia variegata, Ficus carica* and *Solanum nigrum* 200 mg/kg

2.9 Sample collection

Animals were deprived of food throughout the night just after the experimental treatment ended. The next day, the animals were anesthetized and biochemically investigated. Blood was drawn from the retro-orbital plexus of the eye and serum samples were collected by centrifugation. The animals were sacrificed humanely, and the liver removed and washed with chilled physiological solution, and then samples were prepared for the automated determination of oxidative stress markers in the tissue.

2.10 Biochemical Parameters

The biochemical parameters were evaluated to investigate liver function, lipid metabolism, oxidative stress and inflammatory responses. Serum samples were used for the estimation of liver function markers and serum triglycerides, whereas liver tissue homogenates were used for the assessment of oxidative stress markers and inflammatory cytokines.

2.11 Statistical analysis

The mean ± standard error of the mean (SEM) is the data representation for all samples. Analyzing the one way ANOVA, followed by a Dunnett multiple comparison test, statistical comparisons between treatment groups were made against the disease control group. The statistical analysis was performed with the software GraphPad Prism version 8.0.2 from GraphPad Software. $p<0.05$ was considered statistically significant.

3. Results and Discussion

The present study was conducted to investigate the hepatoprotective potential of individual and combined hydroalcoholic extracts of *Bauhinia variegata*, *Ficus carica* and *Solanum nigrum* against CCl_4-induced hepatotoxicity in experimental rats. The hepatoprotective activity was assessed by evaluating changes in body weight, serum biochemical parameters, hepatic oxidative stress markers and inflammatory cytokines. The results demonstrated that treatment with individual plant extracts, particularly their combination, produced improvements in several biochemical and oxidative stress parameters compared with the CCl_4 control group.

Body-weight changes are important indicators of the general health and physiological condition of experimental animals during toxicological investigations. As presented in Table 1**,** the normal control group showed an increase in body weight from 214.60 ± 2.28 g to 230.40 ± 2.63 g during the experimental period. In contrast, the CCl_4 control group exhibited a decrease in body weight from 223.80 ± 1.92 g to 217.60 ± 2.01 g, indicating an adverse effect of CCl_4 administration on the general physiological condition of the animals.

Treatment with individual plant extracts at doses of 100 and 200 mg/kg showed improvements in final body weight compared with the CCl_4 control group. The *Bauhinia variegata* extract-treated groups demonstrated final body weights of 242.70 ± 2.31 g and 249.60 ± 2.44 g, respectively. Similarly, the *Ficus carica*-treated groups showed final body weights of 245.30 ± 2.18 g and 251.90 ± 2.36 g, while the *Solanum nigrum*-treated groups exhibited values of 244.90 ± 2.28 g and 250.70 ± 2.61 g. The combined extract-treated groups showed final body weights of 253.20 ± 2.34 g and 255.40 ± 2.68 g at doses of 100 and 200 mg/kg, respectively. These findings indicate a comparatively greater improvement in body-weight gain following combined extract treatment, particularly at the higher dose. The observed

changes suggest that the combined extracts may help protect against the adverse physiological effects associated with CCl_4 administration.

Serum biochemical parameters are important indicators for evaluating liver function and hepatocellular injury. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are intracellular enzymes that are released into the bloodstream following damage to hepatocytes. Alkaline phosphatase (ALP) is commonly evaluated as an indicator of hepatobiliary dysfunction. The results presented in Table 2 demonstrated considerable alterations in these biochemical parameters in the CCl_4 control group compared with the normal control group.

The ALT level increased from 88.70 ± 7.40 IU/L in the normal control group to 264.30 ± 13.20 IU/L in the CCl_4 control group. Similarly, AST increased from 92.60 ± 9.20 IU/L to 281.80 ± 11.60 IU/L, while ALP increased from 95.80 ± 9.30 IU/L to 241.20 ± 10.70 IU/L. These elevations indicate substantial hepatocellular and hepatobiliary alterations following CCl_4 administration.

Treatment with individual plant extracts resulted in reductions in ALT, AST and ALP levels compared with the CCl_4 control group. Among the individual extracts, *Bauhinia variegata* at 200 mg/kg showed ALT, AST and ALP values of 164.80 ± 8.20 IU/L, 170.80 ± 8.30 IU/L and 162.70 ± 7.60 IU/L, respectively. The corresponding values for *Ficus carica* at 200 mg/kg were 171.40 ± 8.40 IU/L, 179.60 ± 8.70 IU/L and 172.30 ± 8.00 IU/L, whereas *Solanum nigrum* at the same dose showed values of 187.10 ± 8.60 IU/L, 181.70 ± 9.10 IU/L and 169.50 ± 6.90 IU/L.

The combined extract-treated groups demonstrated comparatively greater reductions in these enzyme levels. At 200 mg/kg, the combined extract produced ALT, AST and ALP values of 123.60 ± 6.90 IU/L, 137.80 ± 7.50 IU/L and 140.60 ± 7.40 IU/L, respectively. These results indicate that the combined extracts exhibited a comparatively greater effect on the measured liver enzyme parameters than the individual extracts.

Bilirubin is another important biochemical parameter associated with hepatic function and bilirubin metabolism. As shown in Table 2, the bilirubin level increased from 86.90 ± 5.90 mg/dL in the normal control group to 210.40 ± 8.10 mg/dL in the CCl_4 control group. Treatment with individual and combined plant extracts reduced the reported bilirubin values compared with the toxic control group. The combined extract at 200 mg/kg showed a value of 129.80 ± 6.10 mg/dL, indicating a comparatively greater reduction than the individual extract treatments.

The total protein level decreased from 116.40 ± 10.50 g/dL in the normal control group to 64.90 ± 7.60 g/dL in the CCl_4 control group. Treatment with the combined extracts at 100 and 200 mg/kg increased the reported total protein values to 102.60 ± 8.20 and 106.40 ± 6.90 g/dL, respectively. However, the reported albumin values increased in the CCl_4 control group compared with the normal control group, which is not the usual pattern expected in hepatic synthetic dysfunction. Therefore, the albumin findings require verification before drawing a hepatoprotective conclusion. The serum biochemical findings suggest that the combined plant extracts produced comparatively greater improvements in ALT, AST, ALP, bilirubin and total protein levels.

Oxidative stress plays an important role in the development of CCl_4-induced hepatic injury. CCl_4 undergoes metabolic activation by hepatic cytochrome P450 enzymes, resulting in the formation of reactive free radicals that initiate lipid peroxidation and damage cellular membranes. Malondialdehyde (MDA) is an important indicator of lipid peroxidation, whereas superoxide dismutase (SOD), reduced glutathione (GSH) and catalase (CAT) are important components of the endogenous antioxidant defence system.

As presented in Table 3**,** the MDA level increased from 1.54 ± 0.20 µmol/L in the normal control group to 4.68 ± 0.47 µmol/L in the CCl_4 control group. This elevation indicates increased lipid peroxidation and oxidative damage in hepatic tissue. Treatment with individual plant extracts reduced MDA levels in a dose-related pattern. *Bauhinia variegata* at 200 mg/kg reduced the MDA level to 2.02 ± 0.33 µmol/L, while *Ficus carica* and *Solanum nigrum* at the same dose produced values of 2.56 ± 0.28 and 2.42 ± 0.35 µmol/L, respectively. The combined extract-treated groups demonstrated further reductions, with MDA values of 1.92 ± 0.26 µmol/L at 100 mg/kg and 1.74 ± 0.25 µmol/L at 200 mg/kg. These findings indicate a comparatively greater reduction in lipid peroxidation following combined extract administration.

The SOD level decreased from 136.50 ± 7.60 U/mg in the normal control group to 56.90 ± 11.40 U/mg in the CCl_4 control group. Similarly, GSH and CAT levels decreased from 155.80 ± 7.60 µmol/mg and 104.10 ± 7.60 U/mg to 50.90 ± 11.40 µmol/mg and 43.80 ± 11.40 U/mg, respectively. These changes indicate impairment of the endogenous antioxidant defence system following CCl_4 exposure.

Treatment with individual plant extracts improved the levels of SOD, GSH and CAT compared with the CCl_4 control group. The combined extract at 200 mg/kg produced SOD, GSH and CAT values of 121.60 ± 8.20 U/mg, 146.50 ± 8.00 µmol/mg and 94.60 ± 8.10 U/mg, respectively. These values were comparatively closer to those observed in the normal control and silymarin-treated groups. The improvement in antioxidant enzyme levels, together with the reduction in MDA concentration, suggests that the combined extracts may exert protective effects against oxidative stress-associated hepatic damage.

3.1 Effect of plant extracts on inflammatory cytokines

Inflammatory cytokines play an important role in the progression of hepatic injury. Tumour necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β) and interleukin-6 (IL-6) are important pro-inflammatory mediators involved in the regulation of inflammatory responses. Increased production of these cytokines may contribute to hepatocellular inflammation and tissue damage.

The results presented in Table 4 showed that the levels of TNF-α, IL-1β and IL-6 were considerably increased in the CCl_4 control group compared with the normal control group. TNF-α increased from 20.90 ± 1.30 pg/mL to 84.60 ± 4.40 pg/mL, IL-1β increased from 17.90 ± 1.20 pg/mL to 76.80 ± 4.00 pg/mL, and IL-6 increased from 18.10 ± 1.20 pg/mL to 74.20 ± 4.00 pg/mL. These elevations indicate an enhanced inflammatory response following CCl_4 administration.
Administration of individual plant extracts reduced the levels of all three inflammatory cytokines compared with the CCl_4 control group. A dose-related reduction was observed in the groups treated with *Bauhinia variegata*, *Ficus carica* and *Solanum nigrum*. Among the individual extracts, *Bauhinia variegata* at 200 mg/kg showed TNF-α, IL-1β and IL-6 values of 46.80 ± 2.70, 40.80 ± 2.20 and 43.20 ± 2.30 pg/mL, respectively.

The combined extract demonstrated a comparatively greater reduction in inflammatory cytokine levels. At 200 mg/kg, TNF-α, IL-1β and IL-6 levels decreased to 33.80 ± 2.00, 29.40 ± 1.80 and 30.20 ± 1.70pg/mL, respectively. These findings suggest that the combined plant extracts may help attenuate the inflammatory response associated with CCl_4-induced hepatic injury. The observed reduction in inflammatory cytokines may be associated with the presence of phenolic compounds, flavonoids and other bioactive phytoconstituents in the selected plant extracts. However, further mechanistic studies are required to establish the pathways responsible for these effects.

Table 1: Effect of individual and combined plant extracts on body-weight changes in experimental rats

Group	Treatment	Dose	Initial body weight (g)	Final body weight (g)
I	Normal control	0.9% saline, p.o.	214.60 ± 2.28	230.40 ± 2.63
II	CCl_4 control	1 mL/kg, i.p.	223.80 ± 1.92	217.60 ± 2.01
III	CCl_4 + Silymarin	10 mg/kg, p.o.	237.20 ± 2.41	253.90 ± 2.56
IV	CCl_4 + *B. variegata* extract	100 mg/kg, p.o.	232.40 ± 2.12	242.70 ± 2.31
V	CCl_4 + *B. variegata* extract	200 mg/kg, p.o.	240.30 ± 2.26	249.60 ± 2.44
VI	CCl_4 + *F. carica* extract	100 mg/kg, p.o.	235.50 ± 2.04	245.30 ± 2.18

VII	CCl_4 + *F. carica* extract	200 mg/kg, p.o.	243.10 ± 2.58	251.90 ± 2.36
VIII	CCl_4 + *S. nigrum* extract	100 mg/kg, p.o.	240.10 ± 2.18	244.90 ± 2.28
IX	CCl_4 + *S. nigrum* extract	200 mg/kg, p.o.	246.20 ± 2.47	250.70 ± 2.61
X	Combined plant extracts	100 mg/kg, p.o.	236.40 ± 2.14	253.20 ± 2.34
XI	Combined plant extracts	200 mg/kg, p.o.	247.10 ± 2.36	255.40 ± 2.68

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test.

Table 2: Effect of individual and combined plant extracts on serum biochemical parameters in experimental rats

Group	Treatment	Dose	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Bilirubin (mg/dL)	Albumin (mg/dL)*	Total protein (g/dL)*
I	Normal control	Saline	88.70 ± 7.40	92.60 ± 9.20	95.80 ± 9.30	86.90 ± 5.90	83.40 ± 8.40	116.40 ± 10.50
II	CCl_4 control	1 mL/kg	264.30 ± 13.20	281.80 ± 11.60	241.20 ± 10.70	210.40 ± 8.10	164.20 ± 9.50	64.90 ± 7.60
III	CCl_4 + Silymarin	10 mg/kg	115.80 ± 6.60	129.40 ± 7.20	135.90 ± 5.90	123.80 ± 5.10	91.60 ± 8.50	108.70 ± 6.60
IV	CCl_4 + *B. variegata*	100 mg/kg	181.60 ± 8.90	189.30 ± 8.50	180.40 ± 7.40	171.20 ± 6.90	126.80 ± 7.40	93.80 ± 8.40
V	CCl_4 + *B. variegata*	200 mg/kg	164.80 ± 8.20	170.80 ± 8.30	162.70 ± 7.60	151.90 ± 6.80	111.40 ± 6.60	100.60 ± 7.90
VI	CCl_4 + *F. carica*	100 mg/kg	184.20 ± 9.10	196.20 ± 9.40	184.10 ± 8.40	181.70 ± 7.90	141.80 ± 6.80	82.70 ± 7.00
VII	CCl_4 + *F. carica*	200 mg/kg	171.40 ± 8.40	179.60 ± 8.70	172.30 ± 8.00	166.80 ± 8.20	129.60 ± 7.50	90.60 ± 6.60
VIII	CCl_4 + *S. nigrum*	100 mg/kg	198.60 ± 7.80	192.40 ± 10.10	181.20 ± 7.20	173.90 ± 7.30	142.90 ± 6.70	80.10 ± 7.30
IX	CCl_4 + *S. nigrum*	200 mg/kg	187.10 ± 8.60	181.70 ± 9.10	169.50 ± 6.90	161.80 ± 8.90	131.40 ± 8.40	87.80 ± 6.20
X	Combined plant extracts	100 mg/kg	139.80 ± 7.50	158.90 ± 7.90	152.80 ± 8.20	142.60 ± 5.70	106.20 ± 6.90	102.60 ± 8.20
XI	Combined plant extracts	200 mg/kg	123.60 ± 6.90	137.80 ± 7.50	140.60 ± 7.40	129.80 ± 6.10	96.30 ± 7.60	106.40 ± 6.90

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test.

Table 3: Effect of individual and combined plant extracts on hepatic oxidative stress parameters in experimental rats

Group	Treatment	Dose	MDA (µmol/L)	SOD (U/mg)	GSH (µmol/mg)	CAT (U/mg)
I	Normal control	Saline	1.54 ± 0.20	136.50 ± 7.60	155.80 ± 7.60	104.10 ± 7.60
II	CCl_4 control	1 mL/kg	4.68 ± 0.47	56.90 ± 11.40	50.90 ± 11.40	43.80 ± 11.40
III	CCl_4 + Silymarin	10 mg/kg	1.62 ± 0.23	128.80 ± 6.70	148.20 ± 6.60	98.30 ± 6.60
IV	CCl_4 + *B. variegata*	100 mg/kg	2.38 ± 0.30	74.60 ± 7.90	91.60 ± 7.70	72.50 ± 7.70
V	CCl_4 + *B. variegata*	200 mg/kg	2.02 ± 0.33	93.20 ± 8.90	101.40 ± 9.20	81.60 ± 9.40
VI	CCl_4 + *F. carica*	100 mg/kg	2.90 ± 0.30	80.90 ± 8.00	87.40 ± 7.80	69.90 ± 7.80
VII	CCl_4 + *F. carica*	200 mg/kg	2.56 ± 0.28	92.10 ± 8.60	99.80 ± 8.60	74.20 ± 8.60
VIII	CCl_4 + *S. nigrum*	100 mg/kg	2.74 ± 0.33	84.80 ± 7.50	90.60 ± 7.50	69.10 ± 7.40
IX	CCl_4 + *S. nigrum*	200 mg/kg	2.42 ± 0.35	101.90 ± 8.40	100.20 ± 8.40	82.30 ± 8.50
X	Combined plant extracts	100 mg/kg	1.92 ± 0.26	116.80 ± 6.90	134.60 ± 6.90	86.80 ± 6.90
XI	Combined plant extracts	200 mg/kg	1.74 ± 0.25	121.60 ± 8.20	146.50 ± 8.00	94.60 ± 8.10

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test.

Table 4: Effect of individual and combined plant extracts on inflammatory cytokine levels in experimental rats

Group	Treatment	Dose	TNF-α (pg/mL)	IL-1β (pg/mL)	IL-6 (pg/mL)
I	Normal control	Saline	20.90 ± 1.30	17.90 ± 1.20	18.10 ± 1.20
II	CCl_4 control	1 mL/kg	84.60 ± 4.40	76.80 ± 4.00	74.20 ± 4.00
III	CCl_4 + Silymarin	10 mg/kg	30.40 ± 2.00	25.80 ± 1.70	27.60 ± 1.80
IV	CCl_4 + *B. variegata*	100 mg/kg	55.20 ± 3.00	48.20 ± 2.50	50.40 ± 2.50
V	CCl_4 + *B. variegata*	200 mg/kg	46.80 ± 2.70	40.80 ± 2.20	43.20 ± 2.30
VI	CCl_4 + *F. carica*	100 mg/kg	59.60 ± 3.20	53.90 ± 2.80	54.80 ± 2.70
VII	CCl_4 + *F. carica*	200 mg/kg	51.30 ± 2.80	46.20 ± 2.30	46.70 ± 2.40
VIII	CCl_4 + *S. nigrum*	100 mg/kg	57.80 ± 3.10	52.10 ± 2.60	53.20 ± 2.60
IX	CCl_4 + *S. nigrum*	200 mg/kg	49.40 ± 2.70	44.30 ± 2.40	45.30 ± 2.30
X	Combined plant extracts	100 mg/kg	40.60 ± 2.40	35.60 ± 2.00	37.10 ± 2.00
XI	Combined plant extracts	200 mg/kg	33.80 ± 2.00	29.40 ± 1.80	30.20 ± 1.70

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test.

4. Conclusion

The present study demonstrated the promising hepatoprotective potential of hydroalcoholic extracts of *Bauhinia variegata*, *Ficus carica* and *Solanum nigrum* against CCl_4-induced hepatotoxicity in experimental rats. Treatment with individual plant extracts produced improvements in serum biochemical parameters, antioxidant defence mechanisms and inflammatory cytokine levels compared with the CCl_4 control group. Among the evaluated treatments, the combined extract at 200 mg/kg showed comparatively better protective effects, as evidenced by reduced liver enzyme

levels, lipid peroxidation and pro-inflammatory cytokines, along with improved SOD, GSH, CAT and total protein levels. These findings suggest that the combined extracts may exert hepatoprotective effects through antioxidant and anti-inflammatory mechanisms. However, further studies involving histopathological examination, detailed mechanistic investigations, dose optimization and appropriate statistical validation are necessary to confirm their efficacy and establish their therapeutic potential.

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