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Pharmacological evaluation of the hepatoprotective effects of *Ficus carica* and *Convolvulus prostratus* combination in Carbon Tetrachloride (CCl_4) induced hepatotoxicity in Wistar rats

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ABSTRACT:
This study evaluated the hepatoprotective potential of combined methanolic extracts of *Ficus carica* and *Convolvulus prostratus* against carbon tetrachloride (CCl_4)-induced hepatotoxicity in Wistar rats. Preliminary phytochemical screening revealed the presence of pharmacologically important constituents, including flavonoids, phenolics, alkaloids, terpenoids, glycosides, tannins, and saponins, known for their antioxidant and hepatoprotective activities. Hepatotoxicity was induced by intraperitoneal administration of CCl_4 (1 mL/kg) twice weekly for 21 days, and the combined extract was administered intraperitoneally at doses of 200, 400, and 800 mg/kg body weight, with silymarin (100 mg/kg) serving as the standard reference drug. CCl_4 administration significantly elevated serum SGOT, SGPT, ALP, and bilirubin levels, indicating pronounced hepatocellular injury. Treatment with the combined extract restored these biochemical parameters toward normal values in a dose-dependent manner and normalized both body-weight change and the liver-to-body-weight ratio. Histopathological examination corroborated the biochemical findings, showing reduced necrosis, fatty degeneration, and inflammatory cell infiltration, together with restoration of normal hepatic architecture in the treated groups. The combined extract additionally exhibited concentration-dependent free-radical-scavenging activity in the DPPH assay, supporting its antioxidant potential. The observed hepatoprotective effect is likely attributable to the antioxidant and membrane-stabilizing properties of the phytoconstituents present in both plants. Collectively, these findings indicate that the combined methanolic extracts of *Ficus carica* and *Convolvulus prostratus* possess significant hepatoprotective activity and may represent a safe, effective natural therapeutic option for the management of liver disorders.

1. INTRODUCTION:
The liver is one of the most vital organs of the human body, playing a central role in metabolism, detoxification, digestion, and the maintenance of physiological homeostasis [1]. It contributes to bile production, storage of

glycogen and vitamins, synthesis of plasma proteins, and metabolism of drugs and xenobiotics [2,3,4,7]. Because the liver is the principal site of xenobiotic detoxification, it is particularly vulnerable to injury from drugs, chemicals, and toxic metabolites. Drug-induced hepatotoxicity remains one of the leading causes of acute liver failure worldwide and continues to pose a major challenge in clinical practice [5,6]. Reactive metabolites generated during biotransformation can trigger oxidative stress, inflammation, lipid peroxidation, and hepatocellular necrosis, culminating in severe liver injury [8,9]. Clinically, hepatotoxicity manifests as elevated serum SGOT, SGPT, ALP, and bilirubin levels, together with necrosis, fibrosis, and fatty degeneration of hepatic tissue [9,10].

Oxidative stress plays a pivotal role in the pathogenesis of liver injury by damaging cellular lipids, proteins, and DNA, ultimately leading to mitochondrial dysfunction and hepatocyte death [10]. Antioxidants neutralize free radicals, restore redox balance, and protect hepatic tissue from further damage, making antioxidant-rich medicinal plants promising candidates as hepatoprotective agents [10,12]. Herbal medicines contain pharmacologically important phytoconstituents, such as flavonoids, phenolics, alkaloids, terpenoids, and glycosides, that act synergistically to protect hepatocytes against toxin-induced damage [11,12].

Ficus carica and *Convolvulus prostratus* are traditionally used medicinal plants recognized for their antioxidant, anti-inflammatory, and hepatoprotective properties [13-15]. Experimental studies have demonstrated that these plants reduce oxidative stress, inhibit lipid peroxidation, stabilize hepatocyte membranes, enhance endogenous antioxidant enzyme activity, and normalize liver biomarkers in hepatotoxicity models [16-18]. The present study was therefore designed to evaluate the hepatoprotective potential of a combined extract of *Ficus carica* and *Convolvulus prostratus* against CCl_4-induced hepatotoxicity in Wistar rats.

2. MATERIALS AND METHODS:

Materials:

Plant Material:

Fresh fruits of *Ficus carica* and leaves of *Convolvulus prostratus* were used as plant material, with methanol serving as the extraction solvent. Folin-Ciocalteu reagent, gallic acid, quercetin, aluminium chloride, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were used for phytochemical and antioxidant analyses. Carbon tetrachloride (CCl_4), silymarin, and carboxymethyl cellulose (CMC) were used to induce hepatotoxicity and prepare treatment formulations. Male Wistar rats served as the experimental animals, and ELISA kits for TNF-α, IL-1β, and IL-6 were used to estimate inflammatory markers. Formalin, hematoxylin, and eosin were used for histopathological processing, and a Soxhlet apparatus, rotary evaporator, centrifuge, microscope, and microtome were used throughout the study.

IAEC Approval and Animal Housing

Male Wistar rats weighing 150-250 g were used for the study. Animals were housed in polyacrylic cages under standard laboratory conditions with free access to food and water ad libitum, and were maintained at a room temperature of 22 ± 2°C, relative humidity of 55-60%, and a 12 h light/dark cycle. All experimental procedures were conducted in accordance with CPCSEA guidelines under Institutional Animal Ethics Committee **Registration No. SCOP/IAEC/2025/14.**

Storage of Extract

The dried methanolic extracts of *Ficus carica* fruit and *Convolvulus prostratus* leaves were stored in airtight, amber-colored glass bottles under refrigerated conditions (4 ± 2°C), protected from light and humidity, until further use. Appropriate storage conditions were maintained throughout the study to preserve the stability and integrity of the bioactive constituents.

Experimental Animals

Healthy Male Wistar rats of weighing 150–250 g were procured from the Animal House CAYM Education Trust, Siddhant college of Pharmacy,Talegaon-Chakan Road, A/p Sudumbare, Maval, Pune - 412109, Maharashtra. Animals were maintained under standard laboratory conditions at 25±1 °C with a 12 h light/dark cycle and provided with standard feed and water ad libitum.

Chemicals and Drugs

Carbon tetrachloride (CCl_4) used for induction of hepatotoxicity was procured from Merck Pvt. Ltd. Silymarin used as the standard hepatoprotective drug was obtained from Sigma-Aldrich/Himedia. Biochemical estimation

kits for SGOT, SGPT, ALP, and bilirubin were purchased from ERBA Diagnostics.

Route of Administration
Normal saline, silymarin, and the methanolic extract combination were administered intraperitoneally (i.p.) to ensure rapid systemic absorption. Carbon tetrachloride (CCl_4) was administered orally (p.o.) to induce hepatotoxicity in the experimental animals.

Methods
Phytochemical Screening
The methanolic extracts of Ficus carica and Convolvulus prostratus were subjected to preliminary phytochemical screening using standard qualitative tests [19]. Carbohydrates were identified by the Molisch test, indicated by formation of a purple ring, while alkaloids were confirmed by the yellow precipitate formed in Hager's test. Saponins were detected by persistent foam formation, and tannins were identified by a brownish-green or blue-black coloration with ferric chloride solution [19-21].

Terpenoids and steroids were identified by the Salkowski test, evidenced by a reddish-brown coloration at the interface [19]. Glycosides were detected using the Keller-Killiani test, while phenols and flavonoids were confirmed by ferric chloride and lead acetate tests, respectively [19]. Amino acids were identified by a blue coloration in the Ninhydrin test, and anthraquinones were confirmed by a pink coloration following treatment with benzene and ammonia solution [19].

Preparation of Treatment
For evaluation of hepatoprotective activity, 36 male Wistar rats were randomly divided into six groups of six animals each. Group I (normal control) received normal saline (1 mL/kg). Group II (disease control) received carbon tetrachloride (50% CCl_4 in olive oil) at a dose of 1 mL/kg. Group III received CCl_4 together with the standard drug silymarin (100 mg/kg). Groups IV, V, and VI received CCl_4 in combination with the *Ficus carica-Convolvulus prostratus* (1:1) methanolic extract combination at doses of 200 mg/kg (100 + 100 mg/kg), 400 mg/kg (200 + 200 mg/kg), and 800 mg/kg (400 + 400 mg/kg), respectively. CCl_4 was administered intraperitoneally, and the respective treatments were administered according to the experimental protocol over a period of four weeks.

At the end of the treatment period, animals were fasted overnight and blood samples were collected by retro-orbital plexus puncture. Serum was separated by centrifugation at 3000 rpm for 15 min and used for biochemical analysis, after which liver tissues were isolated for biochemical and histopathological evaluation.

In vivo study
Preparation of CCl_4
Carbon tetrachloride (CCl_4) solution was prepared by mixing equal volumes of CCl_4 and olive oil in a 1:1 (v/v) ratio. The mixture was transferred into a sterile glass container and mixed thoroughly to obtain a homogeneous solution. Fresh solution was prepared on each dosing day and stored in a tightly closed amber-colored container protected from light until use [24].

In-vivo Hepatotoxicity Model
The hepatoprotective activity of the combined methanolic extract was evaluated using CCl_4-induced hepatotoxicity in Wistar rats. Animals were divided into six groups comprising normal control, negative control, three treatment groups (200, 400, and 800 mg/kg), and standard treatment group receiving silymarin (100 mg/kg). CCl_4 was administered intraperitoneally at a dose of 1 mL/kg twice weekly for 21 days to induce hepatotoxicity, while the treatment and standard drugs were administered orally once daily throughout the experimental period [25].

Induction of Hepatotoxicity by CCl_4
Hepatotoxicity was induced in all groups except the normal control group by intraperitoneal administration of CCl_4 mixed with olive oil (1:1 v/v) at a dose of 1 mL/kg body weight twice weekly for 21 days. This repeated administration produced oxidative stress, lipid peroxidation, hepatocellular necrosis, and elevation of liver marker enzymes, thereby mimicking chronic liver injury [25].

Daily Dose of Combined Extract Treatment
The combined methanolic extracts of *Ficus carica* and *Convolvulus prostratus* were administered (intraperitoneal mg/kg, p.o.) was used as the standard hepatoprotective drug. All formulations were prepared freshly in 0.5% CMC before administration, and the dosing volume was adjusted according to the body weight of each animal [25].

Biochemical Analysis
At the end of the experimental period, animals were fasted overnight and blood samples were collected under mild anesthesia by retro-orbital. The blood samples were allowed to clot and centrifuged at 3000 rpm for 15 min to obtain serum. Serum levels of SGOT (AST), SGPT (ALT), ALP, total bilirubin, total protein, and albumin were estimated using standard diagnostic kits according to the manufacturer's instructions. These biochemical parameters were used to evaluate liver function and hepatoprotective activity of the combined extract. Results were expressed as mean ± SEM [24].

Histopathological Study of Liver
Liver tissues were isolated, washed with normal saline, and preserved in formalin solution for histopathological examination. The analysis included evaluation of vascular congestion, hemorrhage, hepatocellular degeneration, vacuolar changes, necrosis, fatty infiltration, and mononuclear cell infiltration in hepatic tissue. Histopathological observations were used to assess the extent of liver damage and protective effect of the treatment [23].

Statistical Analysis
Statistical analysis was performed using GraphPad Prism 10.2.3 software. Data were expressed as mean ± standard deviation (SD) and analyzed using one-way ANOVA followed by Tukey's multiple comparison test. A value of $P < 0.05$ was considered statistically significant.

3. RESULTS:

Phytochemical Screening
The methanolic extracts of *Ficus carica* fruit and *Convolvulus prostratus* leaves were subjected to qualitative phytochemical screening for identification of various bioactive constituents. The results confirmed the presence of carbohydrates, alkaloids, saponins, tannins, terpenoids, steroids, glycosides, phenols, amino acids, flavonoids, and anthraquinones. These phytoconstituents are known to possess antioxidant, anti-inflammatory, membrane-stabilizing, and hepatoprotective properties. In particular, flavonoids and phenolic compounds contribute significantly to free radical scavenging and protection against oxidative stress. The presence of these secondary metabolites supports the potential pharmacological and hepatoprotective activity of the extracts and justifies their further evaluation in experimental studies.

Qualitative Phytochemical Screening of Methanolic Extract

Table 1: Qualitative Phytochemical Screening of Methanolic Extract

Phytochemical	*Ficus carica*	*Convolvulus prostratus*
Alkaloids	+	+
Flavonoids	+	+
Tannins	+	+
Saponins	+	+
Terpenoids	+	+
Glycosides	+	+
Phenols	+	+

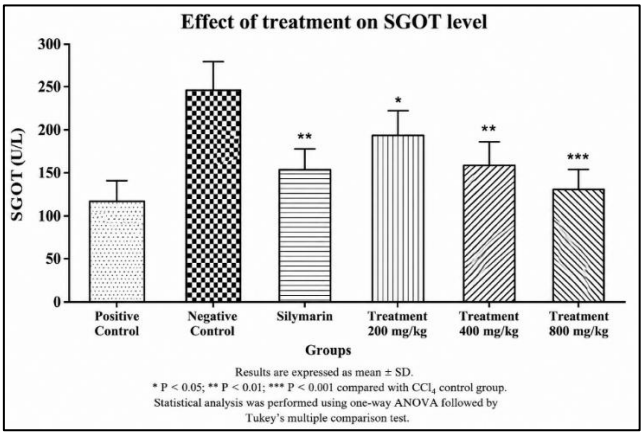
Serum hepatic markers

Table 2: Effect of treatmenton SGOT Level

Group	SGOT (U/L)
Positive Control	118.45
Negative control	245.32
Silymarin	156.2**
Treatment 200 mg/kg	198.4*
Treatment 400 mg/kg	165.22**
Treatment 800 mg/kg	138.6***

Results are expressed as mean ± Standard deviation (SD). $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared with CCl_4 control group. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple

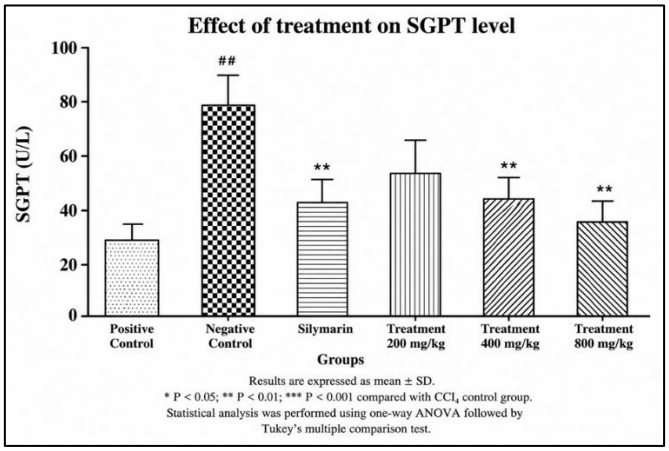
comparison test.



Graph 1: Effect of treatment on SGOT Level

Table 3: Effect of treatment on SGPT Level

Group	SGPT (U/L)
Positive Control	29.14 ± 2.35
Negative control	78.50 ± 8.61##
Silymarin	41.72 ± 6.30**
Treatment 200 mg/kg	52.33 ± 9.15
Treatment 400 mg/kg	44.11 ± 7.26**
Treatment 800 mg/kg	36.50 ± 5.30**



Graph 2: Effect of treatment on SGPT Level

Table 4: Effect of treatment on ALP(U/L) Level

Group	ALP (U/L)
Positive Control	192.60 ± 55.40
Negative control	448.21 ± 70.30##
Silymarin	295.45 ± 62.80**
Treatment 200 mg/kg	348.50 ± 55.40
Treatment 400 mg/kg	301.18 ± 66.50
Treatment 800 mg/kg	265.40 ± 60.20**

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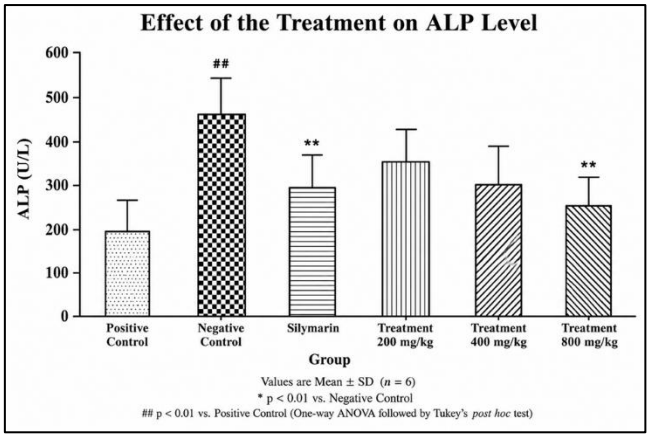
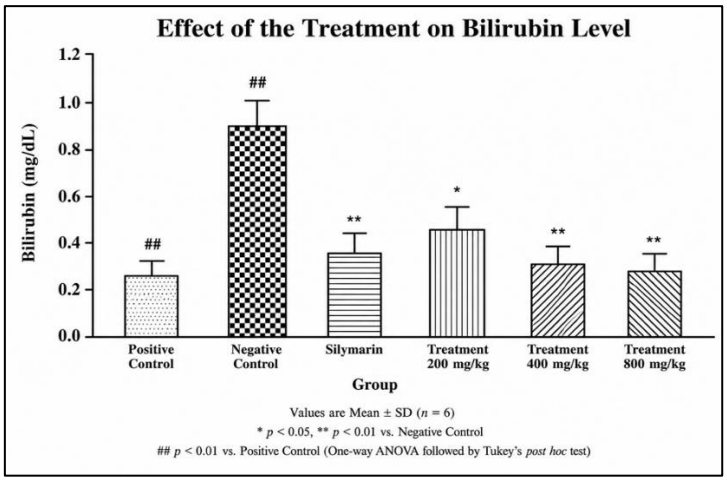


Graph 3: Effect of treatment on ALP Level

Table 5: Effect of the treatment on Bilirubin

Group	Bilirubin (mg/dL)
Positive Control	0.26 ± 0.05
Negative control	0.92 ± 0.08##
Silymarin	0.36 ± 0.09**
Treatment 200 mg/kg	0.44 ± 0.10*
Treatment 400 mg/kg	0.31 ± 0.07**
Treatment 800 mg/kg	0.28 ± 0.06**

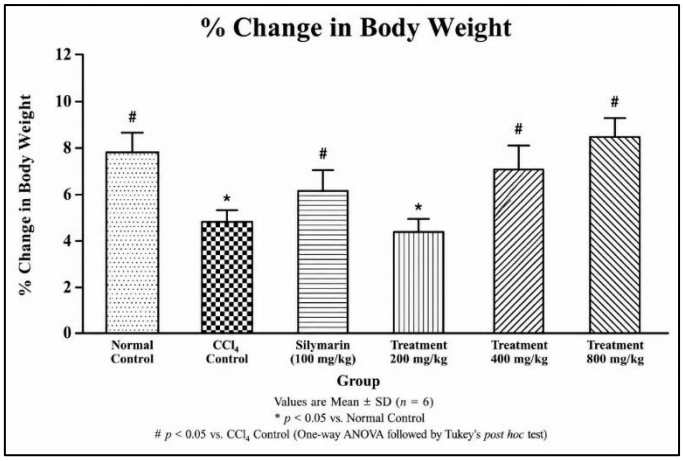


Graph 4: Effect of the treatment on Bilirubin Level

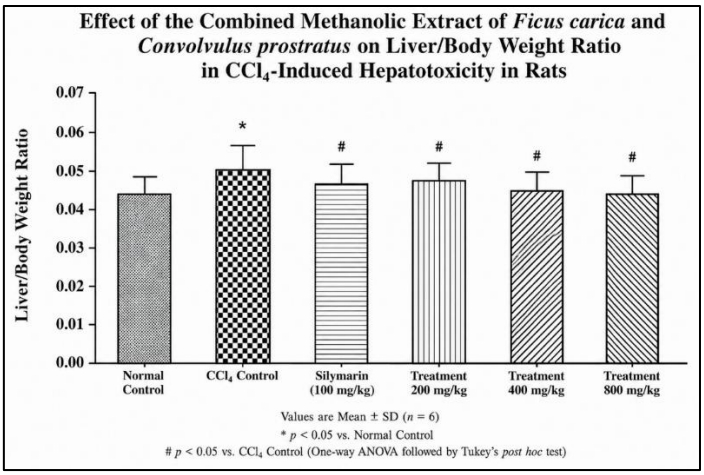
Table 6: Effect of the Combined Methanolic Extract of *Ficus carica* and *Convolvulus prostratus* on % Change in Body Weight and Liver/Body Weight Ratio in CCl_4-Induced Hepatotoxicity in Rats

Groups	% Change in Body Weight	Liver/Body Weight Ratio
Normal Control	7.85 ± 4.90 %	0.0452 ± 0.00120
CCl_4 Control	4.90 ± 15.60 %	0.0510 ± 0.00230 #
Silymarin (100 mg/kg)	6.10 ± 7.95 %	0.0465 ± 0.00140
Treatment 200 mg/kg	4.45 ± 6.85 %	0.0470 ± 0.00260 *
Treatment 400 mg/kg	6.90 ± 9.20 %	0.0460 ± 0.00190 **
Treatment 800 mg/kg	8.50 ± 8.75 %	0.0455 ± 0.00130 **

P < 0.05, ** P < 0.01 compared with the CCl_4 control group.
Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test.



Graph 5: Effect of the Combined Methanolic Extract of *Ficus carica* and *Convolvulus prostratus* on % Change in Body Weight in CCl_4-Induced Hepatotoxicity in Rats.



Graph 6: Effect of the Combined Methanolic Extract of *Ficus carica* and *Convolvulus prostratus* on Liver/Body Weight Ratio in CCl_4-Induced Hepatotoxicity in Rats.

Effect on Liver Histopathology: Histopathological examination supported the biochemical findings. Grossly, livers from the CCl_4-treated group appeared congested and discolored compared with the smooth, uniform appearance of livers from the normal control group (Fig. 1 and Fig. 2). Microscopically, the normal control group showed intact hepatic architecture with normal hepatocytes arranged in cords around the central vein. The CCl_4 control group exhibited extensive hepatocellular degeneration, necrosis, fatty change, inflammatory cell infiltration, and vascular congestion. Groups treated with the standard drug or the combined extract showed a dose-dependent reduction in these pathological changes, with the 800 mg/kg extract group showing near-normal hepatic architecture comparable to the standard treatment group (Fig. 3).

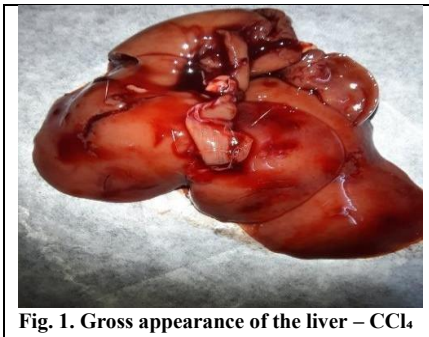


Fig. 1. Gross appearance of the liver – CCl_4 control group

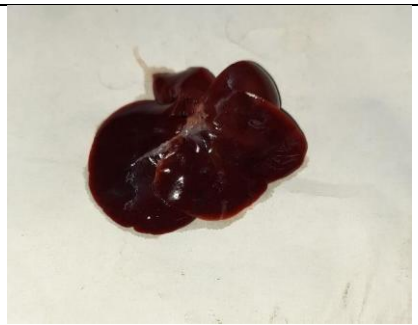


Fig. 2. Gross appearance of the liver – normal control group

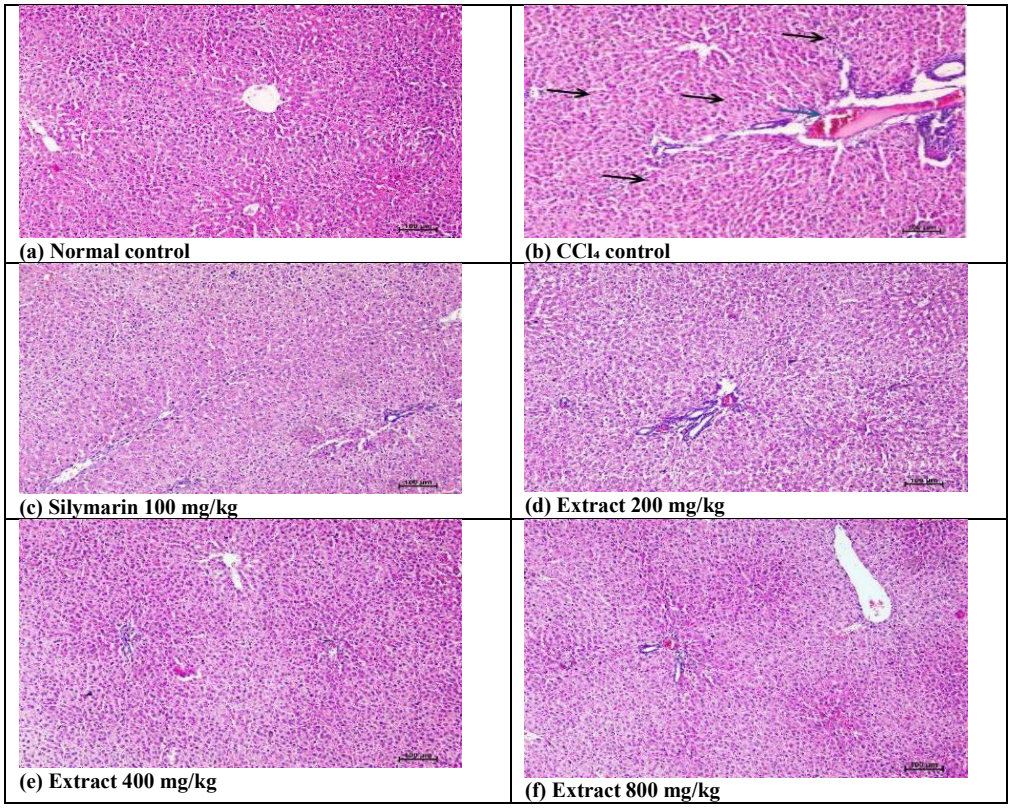


Fig. 3. Photomicrographs of liver sections (H&E stain) showing hepatic architecture across experimental groups (a-f)

Antioxidant Assay:

DPPH Radical Scavenging Assay:

Antioxidant Activity (DPPH Radical Scavenging Assay):

The combined methanolic extract exhibited concentration-dependent free-radical-scavenging activity in the DPPH assay, with percentage inhibition increasing progressively across the tested concentration range (37-99 µg/mL), yielding a linear regression of $y = 0.2399x + 15.442$ ($R^2 = 0.9532$) (Fig. 4). This finding confirms the antioxidant potential of the extract and supports its proposed hepatoprotective mechanism.

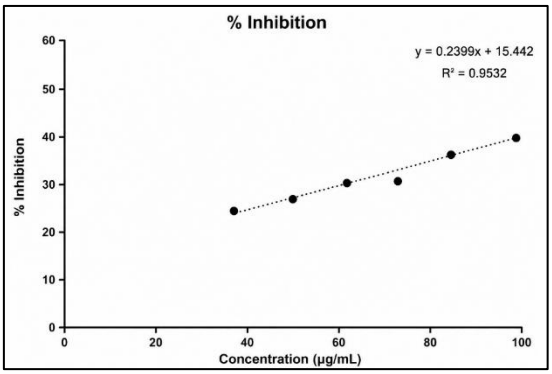


Fig 3 % inhibition VS Combined Methanolic Extract at various concentrations.

Fig. 4. Percentage DPPH radical-scavenging inhibition of the combined methanolic extract at various concentrations

4. DISCUSSION:

The present study evaluated the enhancing hepatoprotective activity of a combined methanolic extract of *Ficus carica* and *Convolvulus prostratus* against CCl_4-induced hepatotoxicity in Wistar rats. Carbon tetrachloride is a well-established hepatotoxicant that produces liver injury through the generation of reactive free radicals, leading to oxidative stress, lipid peroxidation, inflammation, and hepatocellular necrosis. Administration of CCl_4 in the present study caused marked elevation of serum SGOT, SGPT, ALP, and bilirubin, confirming severe hepatic

damage and altered liver function.

Treatment with the combined extract significantly restored these biochemical parameters toward normal levels in a dose-dependent manner. Among the tested doses, the higher doses (400 and 800 mg/kg) exhibited the greatest hepatoprotective activity, comparable to the standard drug silymarin. The reduction in serum enzyme levels indicates stabilization of hepatocyte membranes and prevention of leakage of intracellular enzymes into systemic circulation.

The extract also improved body-weight gain and normalized the liver/body-weight ratio, suggesting recovery from hepatic injury and improvement in overall metabolic status. Histopathological examination further supported the biochemical findings: the CCl_4 control group showed severe hepatocellular degeneration, necrosis, fatty change, inflammatory infiltration, and vascular congestion, whereas extract-treated groups showed restoration of normal hepatic architecture with reduced necrosis and inflammation. The higher-dose group showed near-normal liver histology comparable to the standard-treatment group.

Preliminary phytochemical screening confirmed the presence of flavonoids, phenolics, terpenoids, tannins, alkaloids, and glycosides in both plant extracts. These phytoconstituents possess strong antioxidant and membrane-stabilizing properties that may contribute to free-radical scavenging and protection against oxidative-stress-induced liver injury. The DPPH antioxidant assay further demonstrated significant free-radical-scavenging activity of the combined extract, corroborating its antioxidant-mediated hepatoprotective mechanism.

Overall, these findings suggest that the synergistic combination of *Ficus carica* and *Convolvulus prostratus* possesses significant hepatoprotective potential and may serve as a promising natural therapeutic agent for the management of liver disorders.

5. CONCLUSION:

The present study demonstrated the significant hepatoprotective activity of the combined methanolic extracts of *Ficus carica* and *Convolvulus prostratus* against CCl_4-induced hepatotoxicity in Wistar rats. Administration of CCl_4 produced marked liver damage, as evidenced by elevated serum biochemical markers and oxidative stress. Treatment with the combined extracts significantly restored altered liver parameters, reduced lipid peroxidation, and enhanced endogenous antioxidant enzymes such as SOD, CAT, and GSH, indicating protection against oxidative stress–induced hepatic injury.

The hepatoprotective effect may be attributed to the presence of bioactive phytoconstituents including flavonoids, phenolics, alkaloids, and terpenoids, which possess antioxidant and membrane-stabilizing properties. Histopathological findings further confirmed restoration of normal hepatic architecture and reduction in necrosis and inflammation. Overall, the study suggests that the combined extracts of *Ficus carica* and *Convolvulus prostratus* possess significant hepatoprotective potential and may serve as a safe and effective natural therapy for the management of liver disorders.

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