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Evaluation of Hepatoprotective Activity of *Cornus officinalis* Against CCl_4-Induced Hepatotoxicity in Wistar Rats

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ABSTRACT:
The present study was carried out to evaluate the hepatoprotective activity of Cornus officinalis against carbon tetrachloride (CCl_4)-induced hepatotoxicity in Wistar rats. The fruits of Cornus officinalis were extracted using methanol and subjected to phytochemical and TLC analysis, which confirmed the presence of flavonoids, alkaloids, tannins, glycosides, steroids, and terpenoids. Acute toxicity studies indicated that the extract was safe at lower dose levels. Hepatoprotective activity was assessed using serum biochemical parameters such as SGPT, SGOT, ALP, bilirubin, and albumin along with histopathological examination of liver tissues. Treatment with Cornus officinalis extract significantly restored altered biochemical parameters and reduced hepatic damage caused by CCl_4. Histopathological studies also showed improvement in liver architecture with reduced necrosis and inflammation. The findings suggest that Cornus officinalis possesses significant hepatoprotective activity, possibly due to its antioxidant and bioactive phytoconstituents, and may be useful in the management of liver disorders.

1. INTRODUCTION:
The liver is an essential organ responsible for metabolism, detoxification, secretion, and excretion, and is continuously exposed to environmental pollutants, xenobiotics, alcohol, drugs, and industrial chemicals because of its strategic location in the body. Liver diseases remain a major global health problem and may occur in acute or chronic forms. Acute liver injury can result from alcohol abuse, drugs, poor hygiene, and industrial toxins, whereas chronic liver disorders are commonly associated with hepatitis B and C, cirrhosis, malnutrition, and long-term alcohol consumption [1,2]. Drug-induced hepatotoxicity is one of the major causes of liver failure, accounting for nearly 20–40% of hepatic injury cases. Several drugs including amoxicillin/clavulanate, isoniazid, methimazole, propylthiouracil, and NSAIDs have been associated with severe hepatic damage, leading in some cases to liver transplantation or death [3,4,5].

Alcohol consumption is another major cause of hepatotoxicity. Ethanol metabolism increases the $NADH/NAD^+$ ratio, resulting in triglyceride accumulation and fatty liver (steatosis). Prolonged alcohol intake may further

progress to alcoholic hepatitis and cirrhosis characterized by inflammation, fibrosis, hepatocyte necrosis, and impaired liver function [6]. Viral hepatitis, autoimmune hepatitis, and poor hygiene also contribute significantly to liver disorders worldwide. Hepatitis A infection is closely associated with contaminated food, unsafe water, and poor sanitation [7,8]. Liver dysfunction may further result in complications such as hepatic encephalopathy and jaundice due to accumulation of ammonia and bilirubin in the body [7,9].

Anatomically, the liver is the largest gland of the body situated below the diaphragm in the right hypochondriac region. It receives blood from the hepatic artery and portal vein and performs several vital physiological functions including carbohydrate, protein, and lipid metabolism, detoxification of xenobiotics, bile secretion, storage of vitamins, and synthesis of plasma proteins [10,11]. Hepatotoxicity may occur due to intrinsic toxic substances or idiosyncratic reactions to drugs and chemicals [12,13].

Experimental hepatotoxicity models are widely employed to evaluate the hepatoprotective potential of medicinal agents. Both in vitro and in vivo methods are used for assessing liver injury and protective effects. Commonly used experimental models include carbon tetrachloride (CCl_4), paracetamol, tamoxifen, galactosamine, carbamazepine, and bile duct ligation-induced hepatotoxicity [14,15]. Among these, CCl_4-induced hepatotoxicity is one of the most reliable and extensively used experimental models because it produces oxidative stress, lipid peroxidation, inflammation, and necrosis similar to human liver injury.

Assessment of hepatotoxicity is commonly performed using histopathological examination and biochemical markers such as serum glutamate pyruvate transaminase (SGPT/ALT), serum glutamate oxaloacetate transaminase (SGOT/AST), alkaline phosphatase (ALP), bilirubin, albumin, and lactate dehydrogenase (LDH) [16,17]. In recent years, medicinal plants have gained significant attention for the management of liver disorders due to the lack of completely effective synthetic hepatoprotective drugs. Herbal medicines containing flavonoids, phenolics, tannins, and antioxidants have demonstrated remarkable hepatoprotective activity [18]. Several medicinal plants including *Silybum marianum*, *Tinospora cordifolia*, *Phyllanthus niruri*, *Boerhavia diffusa*, and *Andrographis paniculata* have been scientifically reported for their liver protective effects [19].

Cornus officinalis is a traditional medicinal plant known for its rich content of iridoid glycosides, flavonoids, anthocyanins, and phenolic compounds possessing strong antioxidant and anti-inflammatory properties. Therefore, the present study was designed to evaluate the hepatoprotective activity of *Cornus officinalis* against CCl_4-induced hepatotoxicity in experimental animals.

2. MATERIALS AND METHODS:

Materials

Chemicals and Reagents

The chemicals and reagents used during the study included Methanol (SDFCL), Molisch reagent (OASIS), Mayers reagent (OASIS), Sulfuric acid (SDFCL), Lead acetate (OASIS), Ferric chloride (OASIS), Sodium hydroxide (OASIS), Anesthetic Ether (SDFCL), Rivastigmine (Macleods Pharma), Acetylthiocholine (SDFCL), and DTNB obtained from Sigma. All chemicals and reagents used in the experimental work were of analytical grade.

Apparatus and Instruments

Various instruments and apparatus were used for the experimental study. Weighing balance manufactured by K-roy was used for accurate weighing of chemicals and samples. Magnetic stirrer and cooling centrifuge supplied by Remi were used during preparation and centrifugation procedures. Tissue homogenizer from Prompt was utilized for tissue homogenization. Behavioral studies were carried out using Morris Water Maze apparatus and Elevated Plus Maze apparatus manufactured by K-roy. UV analysis was performed using JASCO V-530 UV-VIS Spectrophotometer. FTIR analysis was conducted using JASCO-460 Plus FT/IR instrument. GC-MS analysis was carried out using Agilent 7890A Gas Chromatography coupled with Agilent 7000B Mass Spectrophotometer (USA). Structural characterization was performed using BRUKER 400 MHz ^1H NMR spectrophotometer.

Ethical Approval

All animals were housed and treated according to the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals). The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (**SCOP/IAEC/2025/13)**

Experimental Animals
Healthy female Sprague-Dawley rats of 8 weeks age weighing between 150–250 g were used for the study. The animals were housed in polypropylene cages fitted with wire mesh tops and maintained with husk bedding under controlled environmental conditions of temperature (25±2°C), relative humidity (60±5%), and a 12 h light/12 h dark cycle. The animals were provided with standard pellet diet and water ad libitum. All experimental procedures were carried out during daytime between 8:00 AM and 4:00 PM.

Methods
Collection and Drying of Plant Material
The fruits of Cornus officinalis were collected from Mahabaleshwar region, Maharashtra, India. The collected plant material was washed, shade dried at room temperature, and coarsely powdered using a mechanical grinder. The powdered material was stored in an airtight container for further study.

Authentication of Plant Material
The plant material was authenticated by Dr. A. R. Yadav, Botanist, Botanical Survey of India, Pune, by comparing its morphological characteristics. A herbarium specimen was deposited at the Botanical Survey of India, Pune, with voucher specimen number ----------

Standardization of Plant Material
Standardization of herbal drugs is essential to ensure the identity, purity, quality, and efficacy of medicinal plants. Variations in climatic conditions, geographical sources, harvesting, and storage may affect the phytochemical constituents of herbal drugs. Therefore, standardization includes evaluation of organoleptic, physicochemical, and phytochemical parameters according to WHO and Indian Pharmacopoeial guidelines. The process also involves assessment of fingerprint profiles, marker compounds, and microbial quality to maintain consistency and safety of herbal formulations [20,21].

Pharmacognostic Study
Macroscopy
Macroscopical evaluation of the plant material was carried out by observing organoleptic characters such as color, odor, taste, size, shape, and surface characteristics for identification and authentication of the crude drug [22].

Microscopy
Microscopical studies were performed according to the method described by Khandelwal (2005). Transverse sections of stem and leaf were prepared, stained with phloroglucinol and hydrochloric acid (1:1), and observed under a microscope at 10X and 45X magnifications [22].

Physical Evaluation
Physical evaluation of crude plant material was carried out to determine its quality, purity, and authenticity according to standard pharmacognostic procedures [23]. The evaluation included determination of foreign organic matter, moisture content, and ash values.

Determination of Foreign Organic Matter
About 5 g of coarsely powdered air-dried drug was spread as a thin layer and examined visually and with a 6X lens for the presence of foreign matter such as dust, stones, insects, and other contaminants. The foreign particles were separated manually and calculated as percentage foreign organic matter [23].

Determination of Moisture Content
Approximately 2 g of powdered drug was accurately weighed in a previously dried and weighed dish and dried in a hot air oven until constant weight was obtained. After cooling in a desiccator, the loss in weight was calculated and expressed as percentage moisture content of the air-dried drug [23].

Determination of Ash Values
Ash values were determined to evaluate the purity and inorganic content of the crude drug [93,94].

Total Ash
About 2 g of air-dried powdered drug was incinerated in a silica crucible at 450°C until carbon-free ash was

obtained. The ash was cooled and weighed, and the percentage of total ash was calculated.

Water-Soluble Ash
The total ash obtained was boiled with 25 mL distilled water, filtered, and the insoluble matter was collected on ashless filter paper. The residue was ignited and weighed. The difference in weight between total ash and insoluble matter represented the water-soluble ash.

Acid-Insoluble Ash
The total ash was boiled with 25 mL hydrochloric acid, filtered, washed with hot water, ignited, cooled, and weighed. The remaining residue was calculated as acid-insoluble ash and expressed as percentage of air-dried drug [23].

Extractive Values
Different extractive values such as water-soluble and alcohol-soluble extractive values were determined according to the standard procedures described in the Indian Pharmacopoeia (1996) [23].

Determination of Water-Soluble Extractive Value
About 5 g of coarsely powdered air-dried drug was macerated with 100 mL of chloroform water in a closed flask for 24 h, with frequent shaking during the first 6 h and allowed to stand for the remaining 18 h. The solution was then filtered, and 25 mL of the filtrate was evaporated to dryness in a shallow dish and dried at 105°C to constant weight. The percentage of water-soluble extractive value was calculated with reference to the air-dried drug [23].

Determination of Alcohol-Soluble Extractive Value
About 5 g of coarsely powdered air-dried drug was macerated with 100 mL of ethanol in a closed flask for 24 h, shaking frequently during the first 6 h and allowing it to stand for 18 h. The mixture was filtered carefully to avoid loss of solvent. Then, 25 mL of the filtrate was evaporated to dryness in a shallow dish, dried at 105°C, and weighed. The percentage of alcohol-soluble extractive value was calculated with reference to the air-dried drug [23].

Extraction
Extraction is an important process used for the separation of active constituents from medicinal plants using suitable solvents. Various extraction methods such as maceration, infusion, digestion, decoction, percolation, fermentation, counter-current extraction, and Soxhlet extraction are commonly employed for isolation of phytoconstituents [24, 25]. Among these methods, Soxhlet extraction is widely used because it provides efficient extraction with less solvent consumption and improved yield.

Soxhlet Extraction Method
The dried and coarsely powdered fruits of Cornus officinalis were subjected to Soxhlet extraction using methanol as solvent. About 500 g of powdered material was packed in a thimble and extracted continuously in a Soxhlet apparatus for 24–48 h until complete extraction was achieved. The extraction process was continued until the solvent in the siphon tube became colorless. The obtained extract was filtered and concentrated under reduced pressure using a rotary vacuum evaporator. The concentrated extract was then dried to obtain a semisolid mass and stored in an airtight container at room temperature for further pharmacological studies [24,25].
Methanol was selected as the extraction solvent because of its ability to dissolve a wide range of polar and moderately polar phytoconstituents such as flavonoids, phenolics, glycosides, tannins, and alkaloids. The percentage yield of extract was calculated with reference to the air-dried powdered drug.

Liquid–Liquid Fractionation with Chloroform and Ethyl Acetate
About 22.8 g of dried hydroalcoholic extract of Cornus officinalis was dissolved in 100 mL of ethanol (1:1) mixture and transferred into a separating funnel [26]. The extract was successively partitioned five times with 50 mL portions of chloroform. The chloroform fractions were collected, combined, and concentrated to obtain a thick paste.

The remaining hydroalcoholic layer was further subjected to successive extraction with ethyl acetate (50 mL × 5). The ethyl acetate fractions were collected, combined, and concentrated to obtain a semisolid mass. The remaining alcoholic fraction was also collected and concentrated separately for further phytochemical and pharmacological

studies.

Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) was carried out for the identification and characterization of phytoconstituents present in the fractions of Cornus officinalis extract [27].

Preparation of TLC Plate

Silica gel-G was used as the adsorbent material. A slurry of silica gel-G was prepared using distilled water and uniformly spread on clean glass plates to obtain a thin layer. The coated plates were allowed to dry at room temperature.

Activation of TLC Plate

The prepared TLC plates were activated by heating in a hot air oven at 105°C for 30 min.

Sample Application and Chamber Saturation

Samples of different fractions were applied as small spots on the activated TLC plates using capillary tubes. The mobile phase was poured into a chromatographic chamber and allowed to saturate for 30 min with the chamber closed properly.

Chromatogram Development

The spotted TLC plate was carefully placed in the chamber containing the mobile phase. The solvent was allowed to ascend up to a distance of approximately 10–15 cm on the silica layer. The developed plates were removed and dried at room temperature.

Visualization

The developed chromatograms were observed visually and under UV light at 254 nm and 365 nm. Suitable spraying reagents such as Vanillin–Sulfuric acid, Methanolic $FeCl_3$ solution, and Anisaldehyde–Sulfuric acid reagent were used for visualization of phytoconstituents. The Rf values were calculated using the standard formula [27].

TLC Characterization of Bioactive Fractions

After pharmacological evaluation, the active fractions were subjected to TLC analysis for identification of phytochemical constituents using different mobile phases and detection methods [24,28].

Sr. No.	Phytoconstituent	Mobile Phase	Detection Method
1	Alkaloids	n-Butanol: Ethyl acetate: Formic acid: Water (30:50:10:10)	UV at 365 nm
2	Glycosides	Ethyl acetate: Methanol: Water (100:16.5:13.5)	UV at 365 nm
3	Flavonoids	Toluene: Ethyl acetate: Glacial acetic acid: Water (100:11:11:26)	Anisaldehyde–Sulfuric acid, UV at 365 nm
4	Tannins	Ethyl acetate: Formic acid: Acetic acid: Water (100:11:11:26)	5% $FeCl_3$ in 0.1 N HCl
5	Steroids	Ethyl acetate: Methanol: Water (70:20:10)	Vanillin–Sulfuric acid
6	Terpenoids and Carotenoids	Cyclohexane: Ethyl acetate (75:25)	UV at 268 nm
		Petroleum ether: Benzene (9:1)	UV at 254 nm
7	Triterpenoids	Chloroform: Glacial acetic acid: Methanol: Water (60:32:12:8)	--
		Ethyl acetate: Glacial acetic acid: Formic acid: Water (100:11:11:26)	Anisaldehyde–Sulfuric acid, UV at 254 nm and 365 nm

Detection of Steroids [80]

Solvent System Used

- Toluene: Ethyl acetate (9:1)
- Ethyl acetate: Methanol: Acetic acid (70:20:10)

Spray Reagents

Vanillin–Sulphuric Acid Reagent

About 0.5 g of vanillin was dissolved in sulphuric acid mixture (40:10). The developed TLC plate was sprayed

with the reagent and heated at 120°C until maximum color intensity was obtained. Blue, blue-violet, or pink colored spots indicated the presence of steroids.

Anisaldehyde–Sulphuric Acid Reagent
About 0.5 mL anisaldehyde was mixed with 10 mL glacial acetic acid, 85 mL methanol, and 5 mL concentrated sulphuric acid. The developed TLC plate was sprayed with the reagent and heated at 100°C for 5–10 min. Blue, blue-violet, or pink colored spots confirmed the presence of steroids.

Detection of Alkaloids [80]
Solvent System Used
- Toluene : Ethyl acetate : Formic acid (50:40:10)

Spray Reagent
Sulphuric Acid Reagent
A 1% solution of concentrated sulphuric acid in ethanol was prepared. The developed TLC plate was sprayed with the reagent and heated at 100°C for 3–5 min. Red-violet or brown colored spots indicated the presence of alkaloids.

Detection of Flavonoids [28]
Solvent System Used
- Toluene: Ethyl acetate: Glacial acetic acid: Water (100:11:11:26)
- n-Butanol: Acetic acid: Water (4:1:5)
- Toluene: Ethyl acetate (9:1)
- Ethyl acetate: Formic acid: Acetic acid: Water (100:11:11:26)

Spray Reagent
Anisaldehyde–Sulphuric Acid Reagent
The developed TLC plate was sprayed with anisaldehyde–sulphuric acid reagent and heated at 100°C for 5–10 min. Yellow-green colored spots indicated the presence of flavonoids.

Detection of Saponins [28]
Solvent System Used
- Toluene: Ethyl acetate (9:1)
- Ethyl acetate: Formic acid: Acetic acid: Water (100:11:11:26)

Spray Reagent
Anisaldehyde–Sulphuric Acid Reagent
The developed TLC plate was sprayed with anisaldehyde–sulphuric acid reagent and heated at 100°C for 5–10 min. Green colored spots confirmed the presence of saponins.

Detection of Tannins [28]
Solvent System Used
- Toluene: Acetone: Ethyl acetate (3:1:2)
- Ethyl acetate: Formic acid: Acetic acid: Water (100:11:11:26)

Spray Reagent
Ferric Chloride Reagent
A 5% ferric chloride solution in 0.1 N HCl was used as spray reagent. The TLC plate was sprayed and heated at 100°C for 5–10 min. Bluish-black colored spots indicated the presence of tannins.

Detection of Glycosides [28]
Solvent System Used
- Ethyl acetate : Methanol : Water (100:16.5:13.5)
- Methanol : Water : Chloroform (35:10:65)

Visualization
The developed TLC plates were observed under UV light at 365 nm, where violet-blue colored spots indicated glycosides.

Spray Reagent

Sodium Nitroprusside Reagent

About 1.5 g sodium nitroprusside was dissolved in 5 mL of 2 N HCl, followed by addition of 95 mL methanol and 10 mL of 25% ammonium hydroxide solution. The solution was filtered and used as spray reagent. Orange-red colored spots indicated the presence of glycosides.

In-vivo Studies

Experimental Animals

Healthy adult Wistar albino rats of either sex weighing 150–250 g were procured from a registered animal house facility. The animals were housed in polypropylene cages under standard laboratory conditions at a temperature of 22 ± 2°C, relative humidity of 50–60%, and a 12 h light/dark cycle. The animals were provided with standard pellet diet and water ad libitum and acclimatized to laboratory conditions for 7 days before the experiment [29,30]. All experimental procedures were carried out according to CPCSEA guidelines after obtaining approval from the Institutional Animal Ethics Committee (IAEC).

Experimental Design

The animals were randomly divided into different groups including normal control, toxic control, standard, and treatment groups, each containing equal numbers of animals [70]. Hepatotoxicity was induced using carbon tetrachloride (CCl_4). The standard group received a standard hepatoprotective drug, while treatment groups received different doses of Cornus officinalis extract. At the end of the treatment period, blood samples were collected for biochemical estimations and liver tissues were isolated for histopathological evaluation.

Dose Selection

The dose of the plant extract was selected based on the results of the acute oral toxicity study. Low and high doses of the extract were selected to evaluate the dose-dependent hepatoprotective activity [29].

Route of Administration

The test extract and standard drug were administered orally using an oral gavage, whereas CCl_4 was administered through the intraperitoneal route according to the standard experimental protocol [29].

Acute Toxicity Study

Acute oral toxicity study of the extract was performed according to OECD guidelines [70]. Experimental animals were fasted overnight and administered a single oral dose of the extract. The animals were observed continuously for the first 4 h and periodically for 24 h for signs of toxicity such as tremors, convulsions, salivation, diarrhea, lethargy, behavioral changes, and mortality. Observations were continued for 14 days to determine the safety profile and selection of suitable doses for further in-vivo studies.

In-vivo Hepatotoxicity Model [29]

The hepatoprotective activity of Cornus officinalis extract was evaluated using a CCl_4-induced hepatotoxicity model in Wistar rats. The animals were divided into normal control, toxic control, standard, and treatment groups. Hepatotoxicity was induced by administration of carbon tetrachloride (CCl_4), which produces liver injury through generation of free radicals and lipid peroxidation. The test extract and standard drug were administered orally for the specified treatment period. At the end of the study, blood samples and liver tissues were collected for biochemical and histopathological evaluation.

Biochemical Parameters [31]

The hepatoprotective activity was assessed by estimating various serum biochemical parameters including serum glutamate pyruvate transaminase (SGPT/ALT), serum glutamate oxaloacetate transaminase (SGOT/AST), alkaline phosphatase (ALP), acid phosphatase (ACP), lactate dehydrogenase (LDH), total bilirubin, and serum albumin. Elevated levels of these enzymes indicate hepatocellular damage due to leakage from injured liver cells, whereas restoration towards normal values indicates hepatoprotective activity of the extract.

Histopathological Study [32,33]

After completion of the experimental study, liver tissues were excised, washed with normal saline, and fixed in 10% neutral buffered formalin for 48 h. The tissues were processed, embedded in paraffin wax, sectioned into thin slices, and stained with hematoxylin and eosin (H&E). The prepared sections were examined under a light

microscope for pathological changes such as necrosis, fatty degeneration, inflammation, and hepatocellular damage to evaluate the protective effect of the extract.

Statistical Analysis

All experimental results were expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. A value of $p < 0.05$ was considered statistically significant.

3. RESULTS

Extraction of Plant Material

The dried powdered fruits of Cornus officinalis were subjected to Soxhlet extraction using methanol as solvent. The obtained extract was concentrated under reduced pressure to obtain a semisolid mass. The percentage yield of the extract was calculated with reference to the air-dried powdered material. The hydroalcoholic extract showed satisfactory yield and was selected for further phytochemical and pharmacological investigations.

Liquid–Liquid Fractionation

The hydroalcoholic extract was subjected to liquid–liquid fractionation using chloroform and ethyl acetate as solvents. The chloroform fraction was obtained as a semisolid mass, while the ethyl acetate fraction yielded a concentrated extract. The remaining hydroalcoholic fraction was also collected separately. All fractions were preserved in airtight containers and used for further TLC characterization and biological evaluation.

Thin Layer Chromatography (TLC) Analysis

Thin layer chromatography of different fractions showed the presence of multiple phytoconstituents. Distinct spots with different Rf values were observed under UV light at 254 nm and 365 nm using various solvent systems. The chromatographic separation confirmed the presence of several bioactive compounds in the extract and fractions of Cornus officinalis.

Identification of Phytoconstituents by TLC

TLC characterization using suitable solvent systems and spray reagents confirmed the presence of various secondary metabolites in the extract. Alkaloids showed reddish-brown spots, flavonoids produced yellow-green spots, tannins exhibited bluish-black spots, glycosides showed violet-blue spots, steroids produced blue or pink colored spots, and saponins appeared as green spots. Terpenoids and triterpenoids were identified by characteristic spots under UV light. The results confirmed that Cornus officinalis contains a wide range of bioactive phytoconstituents responsible for its pharmacological activity.

In-vivo Studies

Acute Toxicity Study

The acute oral toxicity study of Cornus officinalis extract was performed in Wistar rats according to OECD guidelines to evaluate its safety profile and determine the approximate LD_{50} value. The animals were fasted overnight before administration of the extract at different dose levels and observed continuously for the first 4 h and periodically for 24 h for signs of toxicity such as behavioral changes, tremors, convulsions, salivation, diarrhea, lethargy, and mortality. Further observations were continued for 14 days.

At the dose of 2000 mg/kg, toxic symptoms and mortality of two animals were observed on the 5th day, indicating delayed toxicity at higher dose levels. At 1000 mg/kg, mortality of one animal was observed on the 8th day. No mortality or significant toxic manifestations were observed at lower doses, and the animals remained normal throughout the observation period.

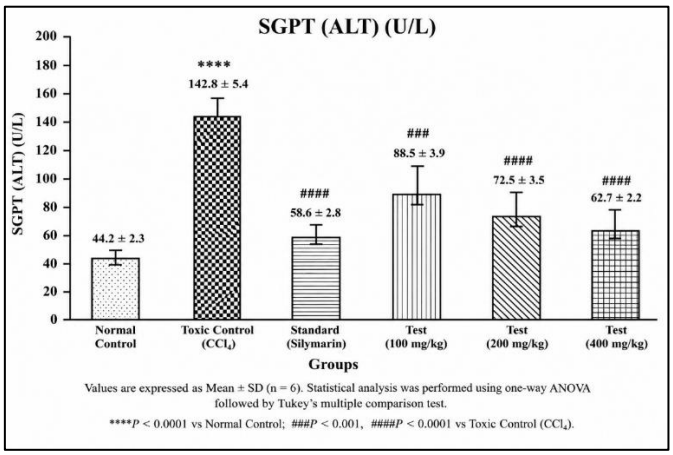
Based on the results, the LD_{50} value of the extract was considered to be above 200 mg/kg and below 2000 mg/kg. Therefore, 200 mg/kg was selected as the safe dose for further in-vivo hepatoprotective studies.

Effect on Biochemical Parameters

Table 1: Effect of *Cornus officinalis* on Biochemical Parameters

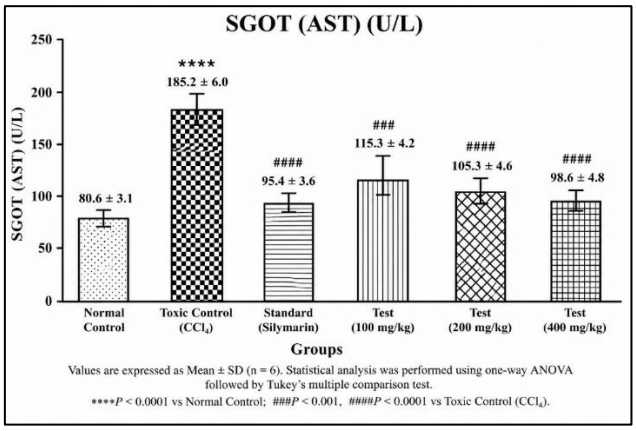
Group	SGPT (ALT) (U/L)	SGOT (AST) (U/L)	ALP (U/L)	LDH (U/L)	Total Bilirubin (mg/dL)
Normal Control	44.2 ± 2.3	80.6 ± 3.1	112.5 ± 4.2	225.3 ± 6.5	0.65 ± 0.04
Toxic Control (CCl_4)	142.8 ± 5.4	185.2 ± 6.0	248.6 ± 7.9	415.7 ± 10.2	2.52 ± 0.10
Standard (Silymarin)	58.6 ± 2.8	95.4 ± 3.6	128.9 ± 4.8	255.2 ± 7.1	0.88 ± 0.05

Test (100 mg/kg)	88.5 ± 3.9	115.3 ± 4.2	158.4 ± 5.6	295.6 ± 8.0	1.30 ± 0.02
Test (200 mg/kg)	72.5 ± 3.5	105.3 ± 4.6	144.6 ± 5.1	276.6 ± 4.3	1.15 ± 0.07
Test (400 mg/kg)	62.7± 2.2	98.6 ± 4.8	130.4 ± 5.6	265.6 ± 8.0	1.03 ± 0.04



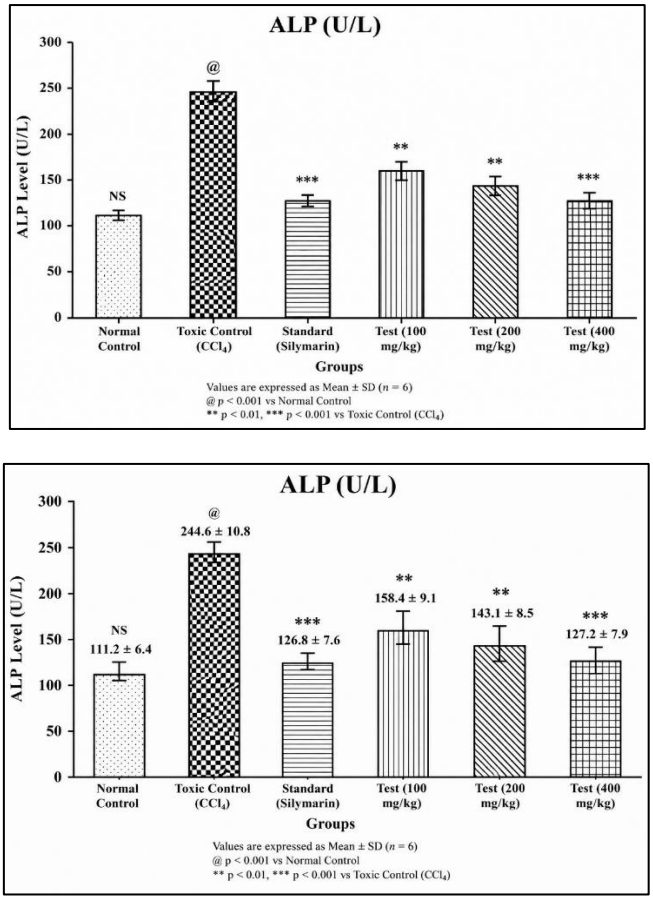
Graph 1. Effect of Treatment on SGPT (ALT) Level

The toxic control (CCl_4) group showed a significant increase in SGPT (ALT) levels (142.8 ± 5.4 U/L) compared with the normal control group (44.2 ± 2.3 U/L, ****P < 0.0001), indicating severe hepatocellular damage. Treatment with Cornus officinalis extract significantly reduced SGPT levels in a dose-dependent manner. The SGPT values decreased to 88.5 ± 3.9 U/L, 72.5 ± 3.5 U/L, and 62.7 ± 2.2 U/L following administration of 100, 200, and 400 mg/kg, respectively. The 400 mg/kg dose exhibited the greatest hepatoprotective effect and showed results comparable to the standard drug Silymarin (58.6 ± 2.8 U/L), demonstrating significant protection against CCl_4-induced liver injury.



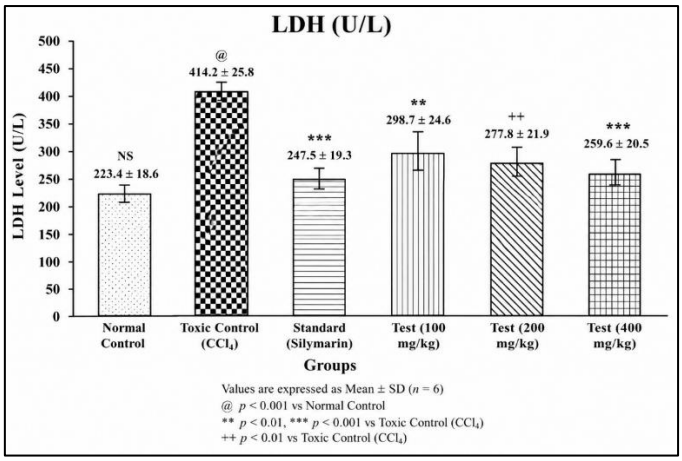
Graph 2: Effect of Treatment on SGOT Level

The CCl_4-treated toxic control group exhibited a significant increase in SGOT (AST) levels (185.2 ± 6.0 U/L) compared with the Normal Control group (80.6 ± 3.1 U/L, ****P < 0.0001), indicating marked hepatocellular damage. Treatment with Cornus officinalis extract significantly reduced SGOT levels in a dose-dependent manner. The SGOT values decreased to 115.3 ± 4.2 U/L, 105.3 ± 4.6 U/L, and 98.6 ± 4.8 U/L following administration of 100, 200, and 400 mg/kg, respectively. The 400 mg/kg dose produced the greatest reduction in SGOT levels and demonstrated hepatoprotective activity comparable to the standard drug Silymarin (95.4 ± 3.6 U/L).



Graph 3: Effect of Treatment on ALP Level

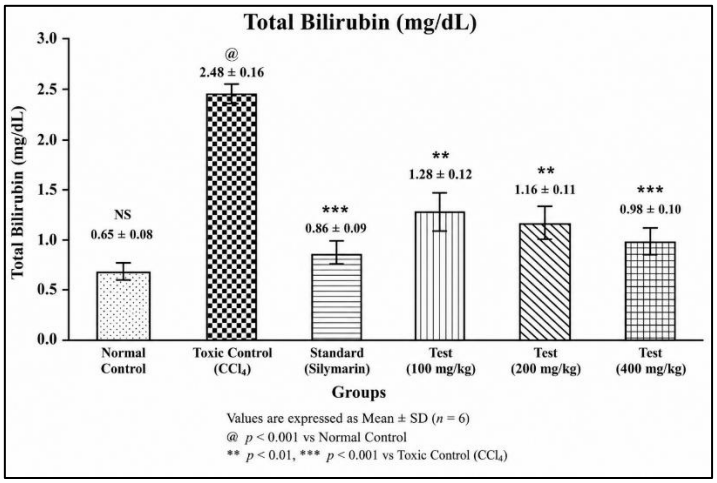
The CCl_4-treated toxic control group showed a significant increase in ALP levels (244.6 ± 10.8 U/L) compared with the Normal Control group (111.2 ± 6.4 U/L, $@P < 0.001$), indicating severe hepatic injury. Treatment with Cornus officinalis extract significantly reduced ALP levels in a dose-dependent manner. The ALP levels decreased to 158.4 ± 9.1 U/L, 143.1 ± 8.5 U/L, and 127.2 ± 7.9 U/L following administration of 100, 200, and 400 mg/kg, respectively. Among the treatment groups, the 400 mg/kg dose produced the greatest reduction in ALP levels and demonstrated hepatoprotective activity comparable to the standard drug Silymarin (126.8 ± 7.6 U/L).



Graph 4: Effect of Treatment on LDH Level

The CCl_4-treated toxic control group exhibited a significant elevation in LDH levels (414.2 ± 25.8 U/L) compared with the Normal Control group (223.4 ± 18.6 U/L, $@P < 0.001$), indicating extensive hepatocellular damage.

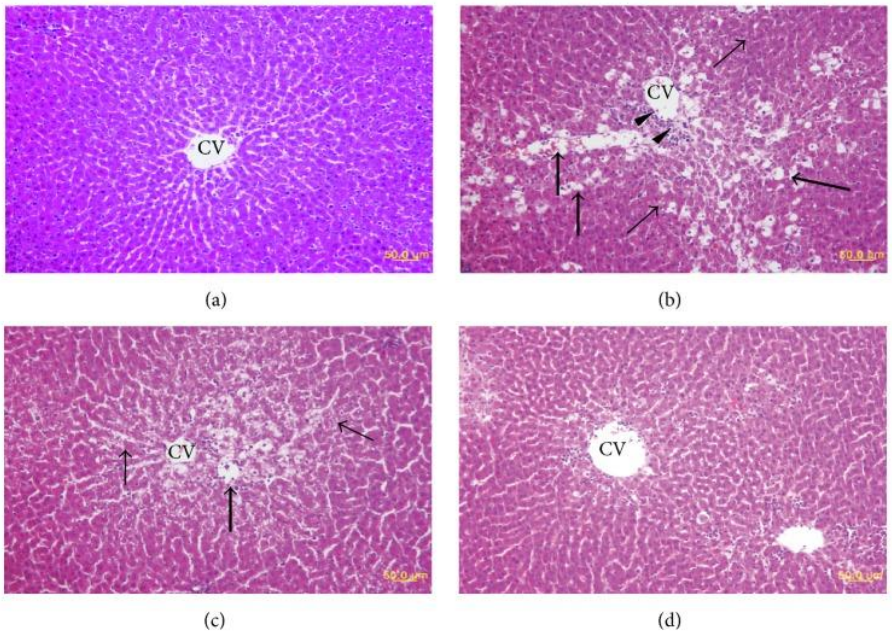
Treatment with Cornus officinalis extract significantly decreased LDH levels in a dose-dependent manner. The LDH values were reduced to 298.7 ± 24.6 U/L, 277.8 ± 21.9 U/L, and 259.6 ± 20.5 U/L following administration of 100, 200, and 400 mg/kg, respectively. The 400 mg/kg dose produced the maximum reduction in LDH levels and exhibited hepatoprotective activity comparable to the standard drug Silymarin (247.5 ± 19.3 U/L).



Graph 5: Effect of Treatment on Bilirubin Level

The CCl_4-treated toxic control group showed a significant increase in total bilirubin levels compared with the Normal Control group (@$P < 0.001$), indicating impaired liver function and reduced hepatic clearance. Treatment with Cornus officinalis extract significantly decreased total bilirubin levels in a dose-dependent manner. The 400 mg/kg dose exhibited the greatest reduction in bilirubin levels and showed hepatoprotective activity comparable to the standard drug Silymarin, demonstrating effective restoration of normal liver function following CCl_4-induced hepatic injury.

Histopathology



A – Normal Control; B – Toxic Control; C – Standard; D – Treatment 400 mg/kg

Histopathological evaluation was performed to assess the extent of liver damage and the hepatoprotective effect of Cornus officinalis extract. Liver tissues were fixed in 10% neutral buffered formalin, embedded in paraffin,

sectioned, and stained with hematoxylin and eosin (H&E) for microscopic examination.
The normal control group showed normal hepatic architecture with well-arranged hepatocytes and intact central vein. The toxic control group exhibited severe necrosis, fatty degeneration, and inflammatory cell infiltration due to CCl_4-induced hepatotoxicity. Treatment with Cornus officinalis extract showed significant restoration of normal liver architecture with reduced necrosis and improved hepatocyte regeneration, confirming its hepatoprotective activity.

4. DISCUSSION:

The present study demonstrated the significant hepatoprotective activity of Cornus officinalis against CCl_4-induced liver damage in Wistar rats. Carbon tetrachloride is a well-established hepatotoxic agent that produces free radicals leading to lipid peroxidation, oxidative stress, necrosis, and inflammatory damage in hepatic tissues. In the present investigation, administration of CCl_4 markedly elevated serum biochemical markers such as SGPT, SGOT, ALP, LDH, and total bilirubin, indicating severe hepatocellular injury and impairment of liver function. Treatment with methanolic extract of Cornus officinalis significantly restored the altered biochemical parameters toward normal values in a dose-dependent manner. The extract at 400 mg/kg showed marked hepatoprotective activity comparable to the standard drug silymarin. Reduction in serum transaminases and bilirubin levels suggests stabilization of hepatocyte membranes and prevention of enzyme leakage from damaged liver cells. Improvement in ALP and LDH levels further confirms restoration of hepatic integrity and functional activity.

Phytochemical screening and TLC analysis confirmed the presence of flavonoids, alkaloids, tannins, glycosides, steroids, and terpenoids in the extract. These phytoconstituents are well known for their antioxidant and free radical scavenging properties. The hepatoprotective effect of Cornus officinalis may therefore be attributed to inhibition of oxidative stress and prevention of lipid peroxidation induced by CCl_4 metabolites.

Histopathological examination further supported the biochemical findings. The toxic control group showed severe fatty degeneration, necrosis, inflammatory cell infiltration, and disruption of normal hepatic architecture. In contrast, extract-treated groups demonstrated marked improvement in liver histology with reduced necrosis, decreased inflammation, and regeneration of hepatocytes. The high-dose treated group showed nearly normal hepatic architecture comparable to the standard group.

Overall, the findings suggest that Cornus officinalis possesses potent hepatoprotective activity due to its antioxidant and cytoprotective phytoconstituents. The study scientifically supports the traditional medicinal use of Cornus officinalis in the management of liver disorders and indicates its potential as a natural hepatoprotective agent.

5.CONCLUSION:

The present study demonstrated that Cornus officinalis possesses significant hepatoprotective activity against CCl_4-induced hepatotoxicity in Wistar rats. Phytochemical screening and TLC analysis confirmed the presence of important bioactive constituents such as flavonoids, alkaloids, tannins, glycosides, steroids, and terpenoids. The extract showed a favorable safety profile in acute toxicity studies and effectively restored altered biochemical parameters towards normal levels. Histopathological evaluation further confirmed the protective effect of the extract by reducing hepatic necrosis, fatty degeneration, and inflammatory changes. Therefore, Cornus officinalis may serve as a promising natural hepatoprotective agent due to its antioxidant and protective properties.

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