



Research Article

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ASSESSMENT OF HEPATOPROTECTIVE AND NEPHROPROTECTIVE EFFECTS OF VITIS VINIFERA LEAF EXTRACT ON CARBON TETRACHLORIDE INDUCED TOXICITY IN RATS

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ABSTRACT

Background: *Vitis vinifera* is known for its antimicrobial activity; however, the hepatoprotective activity of aqueous extracts of aerial parts has also been reported, but the nephroprotective and hepatoprotective activity of ethanolic extracts have not yet been evaluated. **Objective:** To evaluate *Vitis vinifera*'s hepatoprotective and nephroprotective activities against CCl₄-induced toxicity in rats. **Methods:** Two doses of ethanolic extract of *Vitis vinifera* (100 and 200 mg/kg/day) were evaluated and compared with silymarin 100 mg/kg. Biochemical blood parameters like aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), Gamma-glutamyltranspeptidase (GGT), bilirubin, urea, uric acid, total protein and creatinine, and histopathologic changes of liver and kidney were studied and evaluated. **Results:** *Vitis vinifera* reduced the elevated blood levels of ALT, AST, ALP, urea, and creatinine, with the ethanol extract to 200 mg/kg/day being more effective. The histopathologic evaluation suggested that *Vitis vinifera* decreased hepatic and renal necrosis induced by CCl₄. The more significant dose resulted in reductions in AST, ALT, GGT, ALP, and bilirubin of 54, 27, 56, 36, and 17%, respectively. Ethanolic extract 200 mg/kg/day also shows a reduction in elevated levels of Creatinine, Urea, Uric Acid, and Total Protein by 61%, 58%, 29%, and 9%, respectively. **Conclusion:** Hepatoprotective and nephroprotective activities of ethanol extract of *Vitis vinifera* were demonstrated, with ethanol extract to 200 mg/kg/day being the most effective. This presents scientific evidence for using medicinal plants such as *Vitis vinifera* in managing liver and kidney disorders.

INTRODUCTION

Herbal remedies have been utilized for many years in traditional medicine to treat various illnesses. The producers of traditional medicine had an advantage since they were not required to

substantiate statements about the efficacy of their treatments; in contrast, if they were dealing with medication, for example, they would have to offer several pieces of evidence. Certain

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substances included in herbal products may or may not be safe. These studies make certain assumptions, such as that the items might not contain the stated or declared contents or might have come into contact with harmful substances, heavy metals, or narcotics. Additionally, specific historically used herbal remedies may interact with medications in a way that causes significant adverse effects, or they may be deemed harmful for several individuals with particular illnesses [1]. Therefore, any traditional and folk remedy that lacks scientific evidence for its effectiveness and negative effects has to be authorized by science.

The liver is crucial for food digestion, drug processing, detoxification of toxins from the blood, and energy and nutritional restoration. Several standard variables that contribute to liver disease include genetics, obesity, infections, alcohol use, and long-term use of pharmaceuticals such as analgesics, anabolic steroids, antidepressants, and oral contraceptives. In addition, untreated liver diseases can result in liver cirrhosis, a leading cause of mortality [2-3]. Reactive oxygen species are produced in the body due to internal metabolism and exposure to toxins, which causes human oxidative pressure. The kidney is another essential organ for preserving blood volume and electrolyte balance. For our bodies to remain in a state of general haemostasis, kidney function is crucial. This essential organ helps to maintain the balance of numerous critical physiological processes, including detoxification, the control of the hydro-mineral and acid-base balances, the production and regulation of certain hormones, most notably erythropoietin, which is required for the synthesis of hematin, and the regulation of blood pressure [4]. Based on these many roles, mainly the detoxifying function, the kidneys remain the organ most exposed to various xenobiotics in our body. Additionally, several medications were shown to be nephrotoxic in clinical settings [5]. Nephrotoxic medication usage accounts for around 20% of acute renal insufficiencies in hospitalized patients [6].

Numerous antibiotics, including aminoglycosides, tetracyclines, sulfonamides, beta-lactams, fluoroquinolones, vancomycin, and daptomycin, can negatively impact kidney function [7-9]. One of the most common kidney problems resulting from the injection of an external or internal toxicant is nephrotoxicity. Together with vitamin C, the extract from medicinal plants contains triterpenoid and phenolic chemicals. These extracts' antioxidant capacity reduced the oxidative stress brought on by

CCl₄ by scavenging reactive oxygen species and raising the cellular antioxidant index. Additionally, these extracts lessen the inflammation caused by CCl₄ by reducing the expression of NF- κ B and iNOS genes and varying the nitric oxide level [10]. Historically, medicinal herbs have emerged as a more effective and alternative approach for treating kidney stones and urolithiasis, given that most currently available standard therapies are not entirely effective [11], according to Hikino et al. Silymarin is one of the plant extracts that has been studied the most and has a recognized mechanism for treating toxic liver damage orally. Silymarin has been used to treat both acute and chronic liver disorders as a preventive measure [12-13]. Zid reports that it also increases SOD activity, Pietrangelo et al. [14-15] report that it increases glutathione tissue levels, Bosisio et al [16] report that it inhibits lipid peroxidation, Carini et al [17] reports that it enhances hepatocyte protein synthesis, and it supports liver cells through a complex action. The phenolic composition of flavonolignans provides silymarin's antioxidant characteristics, which account for its hepatoprotective action. In order to stop hepatotoxic substances from penetrating hepatocytes, it also promotes liver cell regeneration and stabilizes cell membranes [18-19].

One of the most significant fruit crops worldwide is grapes, *Vitis vinifera* L. (Flame red). They are also eaten as fresh fruits with wine, juice, and other processed goods. The genus *Vitis* has roughly sixty different grape species; the most often-grown variety is *V. vinifera*, sometimes known as European grapes. There are thousands of varieties of *V. vinifera* in existence today. Researchers have analyzed 36 phenolic compounds in the berry samples of 344 representatives of *V. vinifera* varieties to gain an evaluation of the whole spectrum of phytochemicals in grapes, and our results confirmed earlier findings that grapes are rich in phytochemicals [20-22].

It has been intensively researched to show the medicinal benefits of these grape phytochemicals. According to reports, grape extracts have been shown to have antioxidant properties, such as the ability to scavenge free radicals, inhibit lipid oxidation, and reduce the formation of hydroperoxide [23]. They have also slowed aging, reduced plasma oxidative stress, and inhibited specific cancer and cardiovascular diseases [24-26]. Numerous studies have evaluated the antioxidant activities of grape phytochemical compounds but have only examined several cultivars [27-33].

The literature lacks studies on protecting the liver and kidney by *V. vinifera* extracts. The current investigation emphasizes the potential of *V. vinifera* extract to protect the liver and kidneys in animals.

MATERIALS AND METHODS

Collection of Plant Materials

Samples of fresh *Vitis vinifera* (Flame red) aerial parts were taken at random from a Nursery in Bahadurgarh, Haryana, India, and de-leafed in the laboratory. They were then cleaned under running water, dried at room temperature for 8 weeks, and ground into a fine powder. The powder was placed in airtight containers and stored at -20°C until extraction was initiated. The collected specimen was taxonomically authenticated by Chief Scientist Dr. Sunita Garg, RHMD, CSIR-NISCAIR, New Delhi, vide reference no. NISCAIR/RHMD/CONSULT/2022/4076-77

Preparation of Extracts

The Soxhlet extraction method was used to prepare the extract. Ethanol was the best solvent for extracting compounds with antimicrobial activity and antioxidant capacity regardless of the extraction method. Oil from the plant will be extracted in vast quantities by ethanol, which will evaporate entirely. This yields the most products from plants that can be used safely in consumable and food-grade products. One thousand grams of *Vitis vinifera* powdered aerial parts were taken and filled into the porous cellulose thimble. 250 ml of ethanol was added to a round bottom flask, which was attached to a Soxhlet extractor and condenser. After extraction, the extract was evaporated in a vacuum to obtain a dark brown colored viscous residue.

Animals

Wistar albino rats (Male), approximately 150–200 g of around the same age (9–10 weeks), were obtained from the Bilwal Medchem and Research Laboratory Pvt Ltd., Jaipur, Rajasthan. Rats were housed for 5 days before oral administration to allow for their adaptation to the laboratory conditions. The temperature in the animal room was kept at about 25°C and was ventilated with a 12-hour cycle of day and night lights. Water was provided freely to the animals, and a typical meal of rodent pellets. All interventions and animal care procedures were performed in accordance with the ethical guidelines, and the protocol was duly approved by the Institutional Animal Ethics Committee (Reg no.2005/PO/RcBT/S/18/CPCSEA with Protocol Ref No. BMRL/IAEC/2023-18

Hepatoprotective and Nephroprotective properties

Five sets of six male Wistar rats each were put together. Group I was kept intact as the control group and was given normal saline (1 mL, *p.o.*). A single dosage of CCl₄ (1.25 mL/kg body weight) was given to Groups II–V. Group II, the negative control, was given only CCl₄. Ten milligrams per kilogram *p.o.* (20.7 μmol/kg) of silymarin was administered to the third positive control group. 100 and 200 mg/kg of the whole extract of the aerial portions of *V. vinifera* were administered to Groups IV–V. On day twenty-eight, the animals were given CCl₄, and then, 24 hours later, they were sacrificed under mild ether anesthesia. By puncturing the heart, blood samples were obtained, and the serum was separated to assess the biochemical characteristics. A study of sight decided doses of *V. Vinifera* ethanolic extracts, and the purpose was to allow the selection of the appropriate starting dose for the main study.

EXPERIMENTAL GROUPS

Group 1- Control (1 mL, *p.o.*) normal saline solution

Group 2 – CCl₄ (Untreated)

Group 3 - CCl₄ + Silymarin at 10 mg/kg *p.o.* (20.7 μmol/kg, *p.o.*)

Group 4 - CCl₄ + Ethanolic extract of VVL (100mg/kg) (n=6)

Group 5 - CCl₄ + Ethanolic extract of VVL (200mg/kg) (n=6)

Determination of Biochemical Parameters

According to established procedures, the biochemical serum parameters total bilirubin, ALP, GGT, AST, and ALT were measured using the published technique. Serum creatinine, blood urea, and uric acid were also measured [38-40].

HISTOPATHOLOGICAL EVALUATION

Before being examined, each animal's liver and kidney were preserved in 10% formaldehyde for eight days. A section of the organ was sliced to a size of about 6 mm, and it was preserved at 10% in a formaldehyde solution with phosphate buffer. Samples were fixed in paraffin, cleared with xylene, and dehydrated using graded alcohol. Tissue sections 5μm thick were embedded in paraffin wax, sliced, and stained with hematoxylin-eosin. Thin tissue slices were put on permanent slides and sent for histological examination. Cells participating in the inflammatory process (lymphocytes, macrophages, and M2) and neovascularization processes (capillaries and arterioles) are the critical criteria to investigate at the histological level in infarcted tissue.

Statistical Analysis

The means \pm standard errors of means (SEM) are used to express the results. An analysis of variance (ANOVA) in one direction was employed for the statistical analysis. Dunnett's multiple comparisons test was used to further group comparisons once the F-value was statistically significant ($p < 0.05$). SPSS software 17.0 was used for all statistical analyses.

RESULTS AND DISCUSSION

Since the early 1980s, it has been known that extracts from various natural products can protect the liver against CCl₄-induced toxicity at different dosages by decreasing the effects of oxidative stress on liver enzymes. The main reason is that their phytochemicals have an inhibitory effect [41-42]. Because of their antioxidant properties, these phytochemicals may inhibit the microsomal enzymes, limiting the production of free radicals and preventing lipid peroxidation [43]. Additionally, they can promote liver cell regeneration, radical scavenging, and the liver cells' capacity to fight off inflammation brought on by CCl₄ [44]. Carbon tetrachloride is transformed into trichloromethyl free radical CCl₃ in the endoplasmic reticulum and Cl₃COO[•]. With the help of oxygen, these radicals coupled with proteins, cellular lipids, and other macromolecules to cause lipid peroxidation [45]. Transferring hydrogen to free radicals and activating antioxidants is a crucial mechanism of hepatoprotective action. While phenolic compounds, which have a high antioxidant capacity and are present in *V. vinifera*, can be extracted in the greatest amount with ethanol, bioguided phytochemical prospecting is necessary to identify the metabolites responsible for this activity and the mechanism of action through which it exerts its pharmacological action. Additionally, it increases the permeability of cellular membranes to guard against harm from xenobiotics. Additionally, it stimulates DNA polymerase-I to boost the synthesis of ribosomal RNA. Additionally, it acts like a steroid to regulate DNA transcription and stimulate protein synthesis, which results in the regeneration of liver cells. Treatment of the animals with the hepatotoxic agent carbon tetrachloride resulted in a significant increase in transaminases (AST and ALT) and alkaline phosphatase (ALP) levels due to hepatocyte damage [46]. Severe jaundice was reflected by the elevated serum bilirubin levels [47]. Biochemical Parameters for Nephrotoxicity are shown in Table 1. The use of silymarin at a dose of 20.7 μ mol/kg caused a noteworthy decrease ($p < 0.001$) in the elevated biological parameters in rats. Ethanol extract of the aerial portions of *V. Vinifera*, after being injected into rats

(prior to receiving CCl₄), showed a significant ($p < 0.01$; 0.001) reduction in their high bilirubin, AST, ALT, GGT, and ALP levels. The liver histopathology of rats given 200 mg/kg of *V. vinifera* indicated some degree of protection. The nephrotoxicity of CCl₄ is reflected in the elevation of creatinine, urea, uric acid, and total protein serum levels. Results are shown in Table 2. A histological analysis showed that the control group's renal morphology was normal, while the CCl₄-treated group's histopathological changes dramatically differed. Rat kidney cells exposed to 200 mg/kg of *V. vinifera* with CCl₄ exhibited minimal toxicity indicators, such as minor tubule blockage and degeneration.

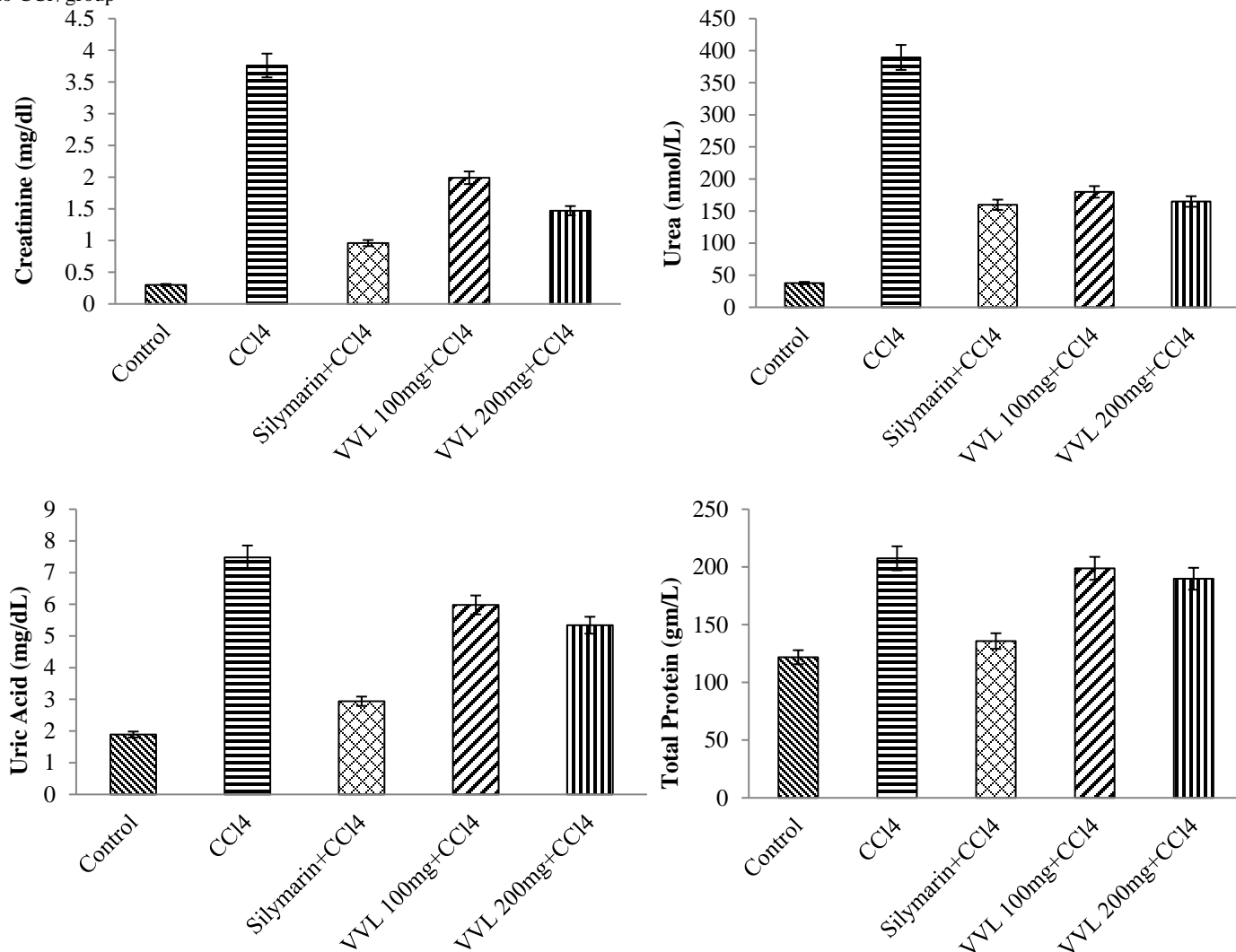
Biochemical Findings for Nephrotoxicity

According to Gowda *et al*, biochemical indicators are crucial for precise diagnosis, risk assessment, and treatment decisions that improve clinical outcomes [48]. A biomarker is an indicator that is objectively assessed and analyzed as a sign of normal biological, pathologic, or pharmacologic reactions to therapeutic intervention, according to the National Institute of Health (NIH) in 2001. The Blood samples were examined for several parameters, including creatinine, total protein, urea, and uric acid, using different groups. One of the most important aspects of evaluating renal function is the measurement of total creatinine. The kidneys are essential for blood filtration, helping to eliminate waste materials like creatinine. A higher-than-normal blood creatinine level may be a sign of compromised renal function. As the tiny standard deviation suggests, Group 1 is the control group, with a mean creatinine level that is relatively low and has limited variability. Depending on the situation, people with reduced muscle mass or those with specific medical disorders may exhibit such low levels. Compared to the other groups, Group 2 (CCl₄), the negative control, had a mean creatinine level that was noticeably higher. When the mean creatinine level falls within this range, it may indicate renal failure or other health problems. Biochemical parameters (estimation of blood) for nephrotoxicity are shown in Figure 1, and the histopathological study of the kidney is depicted in Figure 3. The kidney controls the sodium, potassium, calcium, magnesium, and chloride ions that make up the plasma. According to Pocock and Richards, it eliminates nitrogenous metabolic waste products such as urea, creatinine, and uric acid. Serum electrolyte, urea, and creatinine elevations are trustworthy markers for examining drug-induced nephrotoxicity in humans and animals [49-50].

Table 1: Biochemical Parameters for Nephrotoxicity

Groups Name	Dose (mg/kg)	Estimation of Blood							
		Creatinine (mg/dL)	%	Urea (mg/dL)	%	Uric acid (mg/dL)	%	Total Protein (gm/L)	%
Control	1 mL	0.3±0.05	-	37.7±6.41	-	1.89±0.21	-	121.8±2.58	-
CCl ₄	1.25 mL/kg	3.76±1.25	-	389.50±15.08	-	7.48±0.14	-	207.5±5.79	-
Silymarin	10	0.96±0.06	74	159.8±5.15	59	2.94±0.06	61	135.8±2.14	35
VVL	100	1.99±0.59	47	179.9±10.05	54	5.98±1.05	20	198.8±7.48	4
VVL	200	1.47±0.81	61	164.9±7.79	58	5.34±0.98	29	189.8±3.09	9

Values expressed as mean ± SEM of n = 6. p<0.05; ANOVA, followed by Dunnett's multiple comparison test % Represents % of change respect to CCl₄ group

**Figure 1: Biochemical Parameters (Estimation of Blood) for Nephrotoxicity****Biochemical Parameters for Hepatotoxicity**

Damage to the liver impairs the operation of the hepatocyte transport system, which leads to plasma membrane leakage and elevated blood enzyme levels and tuberculosis. ALP, TB, SGOT and SGPT enzymes are typically eliminated by the liver in bile. Hepatotoxins cause abnormalities in the liver's bile excretion

pathway, which raises blood enzyme levels [51]. Groups 1 to 5 were analyzed for different parameters such as SGOT, SGPT, ALP, Total albumin, Total bilirubin, and total protein. Further investigation is necessary when serum SGOT levels exceed the recommended range. An elevated SGOT may indicate heart problems, liver illness, or muscular injury. While the standard

range for serum SGOT levels varies from lab to lab, it usually ranges from 10 to 40 international units per liter (IU/L).

Increased values are a sign of underlying problems (Clinical Chemistry). Another liver enzyme that is present mainly in the liver is the SGPT. Increased SGPT levels are frequently used to detect liver illnesses, particularly viral hepatitis, as they are a specific sign of liver damage. Usually, the reference range is 7–56 IU/L. The biliary system, bones, and liver all contain the enzyme ALP. Increased ALP values can be a sign of bone or liver illness, as well as bile system blockage. Usually, the reference range is between 30 and 120 IU/L. The liver generates the protein known as albumin, which is a necessary part of blood plasma. Low total albumin levels may indicate malnourishment, renal disease, liver disease, or other illnesses. The usual reference range is 3.4–5.4 mg/dL. The liver processes the yellow pigment called bilirubin, which is created when red blood cells break down. Hemolysis, biliary system blockage, and liver illness can all be indicated by elevated total bilirubin levels. Usually, the reference range is between 0.2 and 1.2 mg/dl. Biochemical Parameters for hepatotoxicity are shown in Figure 2, and the histopathological study of the kidney is depicted in Figure 4. Total protein counts include albumin and globulins to determine the total quantity of protein in the blood. Variations in the overall quantities of protein may indicate problems with the kidneys, liver, or nutrition. Usually, the reference range is between 6.0 and 8.3 mg/dl. Viral infections, heavy drug use, alcohol misuse, and a variety of hazardous substances can all result in liver damage. According to Zhang *et al* and Vladimir-knezevic *et al*, the hepatotoxic experimental model of damage caused by CCl₄ shares many physiological and pathological traits with human hepatotoxic liver injury. According to Popovic *et al*, CCl₄ damages liver through lipid peroxidation, oxidative stress and inflammation. The CYP2E1 enzyme breaks down CCl₄ in the liver to produce the hazardous reactive

trichloromethyl and trichloromethyl peroxide radicals [52-55]. These reactive radicals also attach to unsaturated fatty acids in the membranes of mitochondria, endoplasmic reticulum, and hepatocytes. This results in a chain lipid peroxidation process that damages and eventually kills intracellular structures and hepatocytes [54].

HISTOPATHOLOGICAL EVALUATION

The histopathological investigations provide proof of the medication's effectiveness as a protector. When the control kidney was examined under a microscope, both cortexes and the renal corpuscle seemed normal. They were spherical, compact formations with a thin renal or Bowman's space around the glomerulus. Conversely, the kidneys of rats on a high-casein diet showed both cortical and medullary alterations, such as the glomerulus shrinking in rats that left the vast Bowman's gap in the experimental animals. Rats also showed localised tubular epithelial cell degenerations, partial thickening of the capsule and the appearance of tubular casts in the ducts, loop of henle, and cortical and medullary tubules.

Rats treated with *Vitis vinifera* concurrently show less hepatic cell damage than those treated with silymarin alone. Although they are injured, intralobular veins are not as much. The endothelium is occasionally disturbed. Atrophy is seen in the hepatic cells next to the intralobular vein. Greater hepatoprotective action is seen in the liver segments administered *Vitis vinifera*. A small number of hepatocytes in the immediate area of the intralobular vein show almost little damage. All around, the endothelium lining is smooth, save for one or two areas. Hepatocytes seem normal; only a small percentage of cells have more vacuoles in their cytoplasm. The findings of the biochemical parameters are confirmed by the histological investigation.

Table 2: Biochemical Parameters for Hepatotoxicity

Groups Name	Dose (mg/kg)	Parameters (blood serum)									
		AST (IU/L)	%	ALT/SGOT (IU/L)	%	GGT (IU/L)	%	ALP/SGPT (IU/L)	%	Bilirubin (mg/dL)	%
Control	1 mL	99.8±3.54	-	29.8±2.95	-	2.8±7.31	-	62.9±0.08	-	0.4±0.03	-
CCl ₄	1.25 mL/kg	312.7±8.05	-	173.74±7.15	-	19.6±4.35	-	364.43±5.85	-	2.34±0.08	-
Silymarin	10	124.95±2.59	60	63.25±5.85	63	4.55±1.86	77	115.33±0.58	68	0.79±0.02	66
VVL	100	200.3±6.89	36	99.55±6.18	43	8.89±2.14	54	273.32±0.05	25	1.72±0.08	26
VVL	200	295.7±3.56	54	127.22±3.19	27	8.55±1.08	56	231.8±0.005	36	1.95±0.07	17

Values expressed as mean ± SEM of n = 6. p<0.05; ANOVA, followed by Dunnett's multiple comparison test AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyltranspeptidase; ALP: alkaline phosphatase.

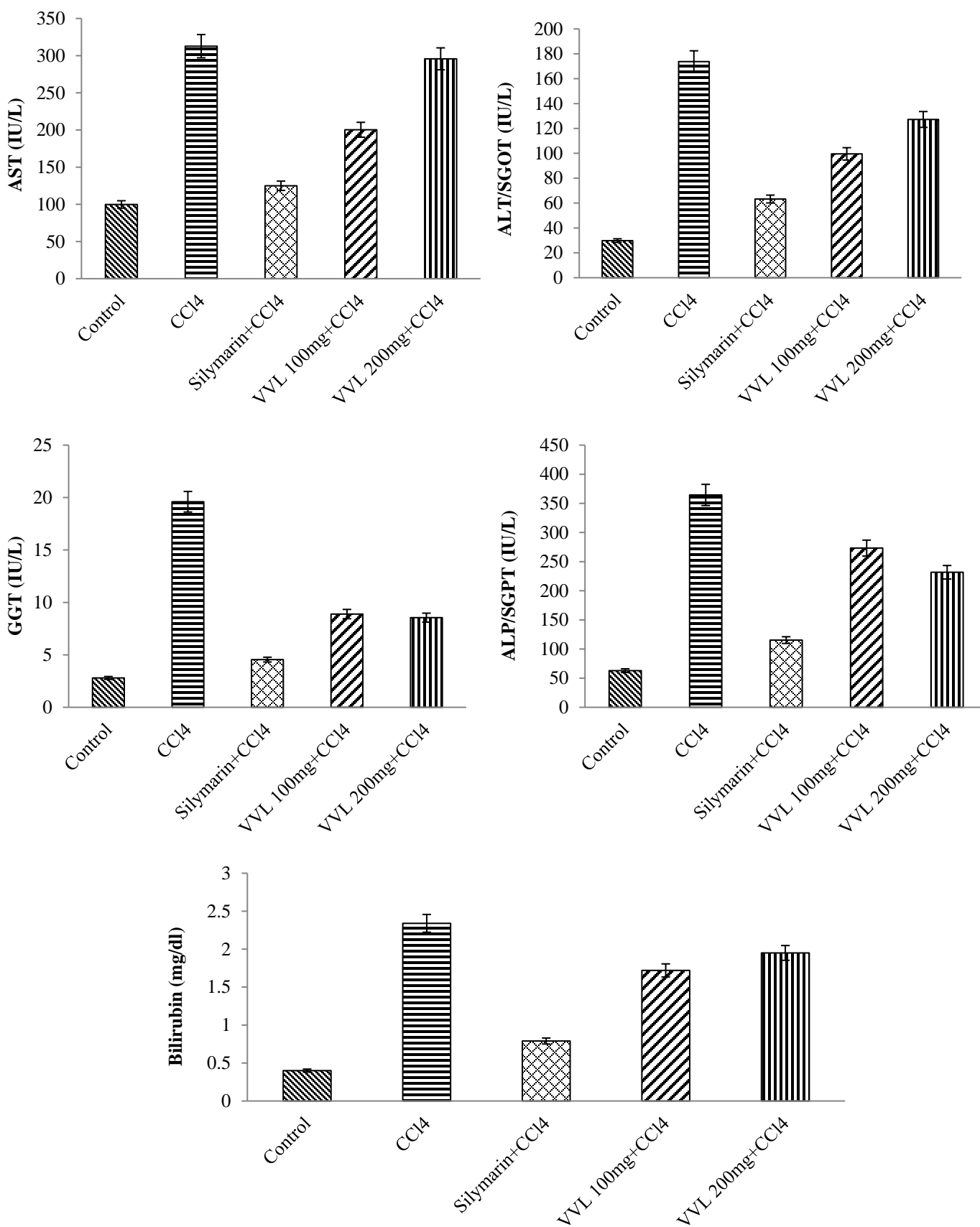


Figure 2: Biochemical Parameters for Hepatotoxicity

CONCLUSION

An overview of the main conclusions and implications of the research study of hepatoprotective and renoprotective effects of *Vitis Vinifera* (Aerial Parts) on carbon tetrachloride-induced toxicity in rats might be found in the study's conclusion. Two different doses of ethanolic extract of *Vitis vinifera* (100 and 200 mg/kg/day) were evaluated and compared with silymarin 100 mg/kg. Rats received these doses for 5 days, and on the 3rd and 4th day, CCl₄ (50 mg/kg i.p.) was administered 1 h after treatment. Animals were sacrificed 48 hours after the last injection of CCl₄.

Biochemical blood parameters and histopathologic changes in the liver and kidney were studied and evaluated. Extracts reduced the elevated blood levels of ALT, AST, ALP, urea, and creatinine, with the ethanol extract to 200 mg/kg/day being more effective. The histopathologic evaluation suggested that *Vitis vinifera* decreased hepatic and renal necrosis induced by CCl₄. According to our research, *Vitis vinifera* antioxidant qualities may have something to do with the reported protective benefits. It improves the antioxidant defense systems within cells, lowering oxidative stress in the kidneys and liver. The results of this study have consequences for kidney and liver health, especially when it comes to damage caused by toxins. Extract from *Vitis vinifera* may offer therapeutic benefits in defending against toxic attacks on these important organs. Further study of the extract fractions and components is highly recommended. To sum up, our research proves *Vitis vinifera*'s hepatoprotective and renoprotective properties (aerial portions) in a rat model of contamination caused by carbon tetrachloride.

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Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest

AUTHOR CONTRIBUTION

All authors contributed to the study's conception and design. Pragi collected data results. Ashok Kumar performed an analysis. Amit Sharma and Varun Kumar wrote the first draft of the manuscript, and all authors corrected and updated previous versions. All authors gave final approval.

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