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EVALUATION OF THE HEPATOPROTECTIVE AND NEPHROPROTECTIVE PROPERTIES OF BAEL FRUIT EXTRACT AGAINST CARBON TETRACHLORIDE-INDUCED TOXICITY IN RATS

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ABSTRACT

Background: *Bael* is well-known for its antibacterial properties. Aqueous fruit extracts have also been shown to have hepatoprotective properties; the nephroprotective and hepatoprotective properties of Ethanolic extracts have not yet been tested. **Objective:** To evaluate the hepatoprotective and nephroprotective activities of *Bael* against CCl_4-induced toxicity in rats. **Methods:** Two dosages of *Bael's* Ethanolic extract (100 and 200 mg/kg/day) were compared with 100 mg/kg of silymarin. Histopathologic alterations of the liver and kidney, as well as biochemical blood parameters such as bilirubin, urea, uric acid, total protein and creatinine, alkaline phosphatase (ALP), alanine aminotransferase (ALT), were examined and assessed. **Results:** *Bael* was more successful in lowering high levels of urea, creatinine, ALT, AST, and ALP when he used a 200 mg/kg/day methanol extract. According to the histopathologic assessment, Bael lessened the CCl4-induced hepatic and renal necrosis. The more significant dose resulted in reductions in AST, ALT, GGT, ALP, and bilirubin of 45,25, 52,36, and 16%, respectively. Ethanolic extract 200 mg/kg/day also shows a reduction in elevated levels of Creatinine, Urea, Uric Acid, and Total Protein by 57%, 52%, 34%, and 9%, respectively. **Conclusion:** There were established hepatoprotective and nephroprotective effects of the *Bael* fruit methanol extract, with 200 mg/kg/day being the most beneficial dose. This provides scientific proof that medicinal herbs like Bael can be used to treat renal and liver diseases.

INTRODUCTION

Plant materials or their extracts have been utilized as drugs in many parts of the world, India and China being the oldest among them. The Indian subcontinent has one of the richest expertise in traditional medicine. Every year, more than five million children under the age of five die from a severe diarrhea disease, classifying diarrhea as the most important cause of childhood mortality in developing countries. These studies make Certain assumptions, such as the possibility that the objects don't contain the declared or stated contents or that they have come into touch with dangerous materials, heavy metals, or drugs. Furthermore,

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certain traditionally used herbal medicines might have dangerous interactions with drugs or be considered toxic for certain persons with specific conditions [1]. Therefore, science must approve any traditional or folk cure not supported by data regarding its efficacy or side effects.

The liver regulates food digestion, medication processing, and blood toxin clearance and replenishes nutrition and energy. Genetics, obesity, infections, alcohol consumption, and prolonged use of medications such as analgesics, anabolic steroids, antidepressants, and oral contraceptives are some of the major factors that lead to liver disease.

Additionally, liver cirrhosis, a major cause of death, can arise from untreated liver disorders [2–3]. Humans experience oxidative pressure from the body's production of reactive oxygen species due to internal metabolism and exposure to pollutants. The kidney is another vital organ to maintain blood volume and electrolyte balance. Kidney function is essential for our bodies to maintain a condition of general hemostasis.

This vital organ supports the homeostasis of many vital physiological functions, such as detoxification, the regulation of the hydro-mineral and acid-base balances, the synthesis and control of specific hormones, most notably erythropoietin, which is necessary for the synthesis of hematite, and blood pressure regulation [4]. Owing to their numerous functions, especially detoxifying, the kidneys remain the organ in our body most exposed to different xenobiotics. Furthermore, several drugs have been demonstrated to be nephrotoxic in clinical contexts [5]. 20% or more of hospitalized patients' acute renal insufficiencies are caused by the use of nephrotoxic medications [6].

Various antibiotics, such as aminoglycosides, tetracyclines, sulfonamides, beta-lactams, fluoroquinolones, vancomycin, and daptomycin, might adversely affect kidney function [7-9]. Nephrotoxicity is one of the most prevalent kidney issues brought on by the injection of an internal or external toxicant. The extract from medicinal plants contains phenolic and triterpenoid compounds and vitamin C. The antioxidant ability of these extracts raised the cellular antioxidant index and scavenged reactive oxygen species, thereby mitigating the oxidative stress caused by CCl_4. Furthermore, by altering the nitric oxide level and lowering the expression of the NF-κB and iNOS genes, these extracts decrease the inflammation brought on by CCl_4 [10].

There are some limitations to the modern treatment strategy, but medicinal herbs are more successful and alternative strategies for treating kidney stones and urolithiasis [11]. According to Hahn G. et al., silymarin is among the plant extracts that have been researched the most and has a recognized mechanism of action for treating toxic liver damage orally. As a prophylactic, silymarin has been utilized to treat acute and chronic liver diseases [12–13].

Abou Zid reports that it helps to raise the SOD activity, Surai PF et al. [14-15] reports that it raises the levels of glutathione, Zhao J *et al.* [16] explain that it helps in the inhibition of lipid peroxidation, Saller R *et al.* [17] reports that it increases the 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. To stop hepatotoxic substances from penetrating hepatocytes, it promotes liver cell regeneration and stabilizes cell membranes [18-19].

Bael is a plant used in the worship of lord Shiva. Widely. This plant also has many medicinal activities. Some important mother plants or cultivars have been selected in India and Sri Lanka. Researchers have analyzed more than 18 phenolic compounds in the Bael samples Bael to gain an evaluation of the whole spectrum of phytochemicals in Bael, and our results confirmed earlier findings that grapes are rich in phytochemicals. The primary phytochemicals exhibiting bioactivity are mamelon, marmalade, and graphene, which are present in fully ripe fruit. Previous studies have demonstrated that these compounds significantly affect hepatoprotective activity. [20-22].

Extensive studies have been conducted to demonstrate the medical effects of these chemical constituents present in Bael. As per the findings, Bael extracts have demonstrated antioxidant characteristics, including the capacity to scavenge free radicals, impede lipid oxidation, and diminish hydroperoxide production [23]. They have also been shown to slow aging, reduce plasma oxidative stress, and inhibit certain cancers and cardiovascular diseases [24-26]. Numerous studies have evaluated the antioxidant activities of Bael phytochemical compounds but have only examined a small number of cultivars [27]. The

literature is deficient in studies on liver and kidney protection by Bael extracts. The current investigation emphasizes the potential of *Bael* fruit for protecting the liver and kidneys in animals.

MATERIALS AND METHODS

Collection of Plant Materials

Fresh Bale fruit samples were randomly selected from a market in Bahadurgarh, Haryana, India. They were then cleaned under running water, dried for eight weeks at room temperature, and ground into a fine powder. The powder was sealed in airtight containers and kept at -20 °C until the extraction process began. The Dr. YS Parmar University of Horticulture and Forestry in Nauni Solan, Himachal Pradesh, India, provided taxonomy authentication for the obtained specimen (ref. number UHF-Harbarium no. 13957 on 12/4/2022).

Preparation of Extracts

The extract was prepared using the Soxhlet extraction method. One thousand grams of Bael fruit powdered were obtained. Following extraction, the extract was vacuum-evaporated to produce a viscous residue with a dark brown colour.

Animals

Male Wistar albino rats weighing between 150 and 200 g and roughly 9 to 10 weeks old were purchased from Maharishi Markenshwar University Ambala. The animals were kept in regulated conditions with 12/12 hours of light and darkness, a temperature of 22 ± 2°C, and a humidity of 55%. Drinking water was available to them at all times. Every experiment was carried out following moral principles. The institutional Animal Ethics Committee Formally authorized the protocol (Reg no. 1355/PO/Re/S/10/CPCSEA with Protocol Ref. Number. MMCP-IAEC-192), and all the interventions and animal care procedures were carried out in compliance with the ethical norms.

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 Bael *fruit* were administered to Groups IV–V. On day twenty-eight, the animals were given CCl4, and then, 24 hours later, they were put to death under ether anesthesia. By puncturing the heart, blood samples were obtained, and the serum was separated to assess the biochemical characteristics. The sighting study aimed to enable the selection of a suitable starting dose for the main study. The doses of bael (*Aegle marmelos*) ethanolic extracts were determined using this method.

EXPERIMENTAL GROUPS

Group 1- Control (1 ml, *p.o.*) normal saline solution
Group 2 – CCl4 (Untreated)
Group 3 - CCl4 + Silymarin at a dose of 10 mg/kg *p.o.* (20.7 µmol/kg, *p.o.*)
Group 4 - CCl4 + Ethanolic extract of AM (100mg/kg) (n=6)
Group 5 - CCl4 + Ethanolic extract of AM (200mg/kg) (n=6)

Determination of Biochemical Parameters

The biochemical serum parameters alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltranspeptidase (GGT), total bilirubin, and alkaline phosphatase (ALP) were assessed using the published technique in accordance with recognized procedures. Blood urea, uric acid, and serum creatinine were also measured [28-30].

HISTOPATHOLOGICAL EVALUATION

The liver and kidney of each animal were kept in 10% formaldehyde for eight days before examination. An organ segment measuring approximately 6 mm long was cut and preserved in 10% formaldehyde solution with phosphate buffer. The samples underwent paraffin fixation, xylene clearing, and graded alcohol dehydration. Haematoxylin-eosin staining was applied to 5µm thick tissue sections embedded in paraffin wax, cut, and examined. Thin tissue slices were sent for histological analysis after being mounted on permanent slides. The key factors to look into at the histological level in infracted tissue include cells involved in the inflammatory process (lymphocytes, macrophages, and M2) and neovascularisation processes (capillaries and arterioles).

Statistical Analysis

The data are expressed as means ± standard errors of means (SEM). An analysis of variance (ANOVA) in one direction was used for the statistical analysis. After the F-value was statistically significant ($p<0.05$), group comparisons were

conducted again using Dunnett's multiple comparisons test. All statistical analyses were performed using SPSS software version 17.0.

RESULTS AND DISCUSSION

Hepatocyte injury was indicated by a significant increase in alkaline phosphatase (ALP) and transaminases (AST and ALT) levels when the animals were given the hepatotoxic chemical carbon tetrachloride. Elevated serum bilirubin levels were indicative of severe jaundice, which was the manifestation of this injury. Biochemical Parameters for Nephrotoxicity are shown in Table 1. The use of silymarin at a dose of 20.7 µmol/kg caused a noteworthy decrease ($p<0.001$) in the elevated biological parameters in rats. Ethanolic extract of *Bael fruit,* after being injected into rats (before receiving CCl_4), 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 *Bael* indicated some protection. The nephrotoxicity of CCl_4 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 *Bael* with CCl4 exhibited minimal toxicity indicators, such as minor tubule blockage and degeneration.

Biochemical Findings for Nephrotoxicity

According to Kirtane et al., biochemical indicators are crucial for precise diagnosis, risk assessment, and treatment decisions that improve clinical outcomes [31]. 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.

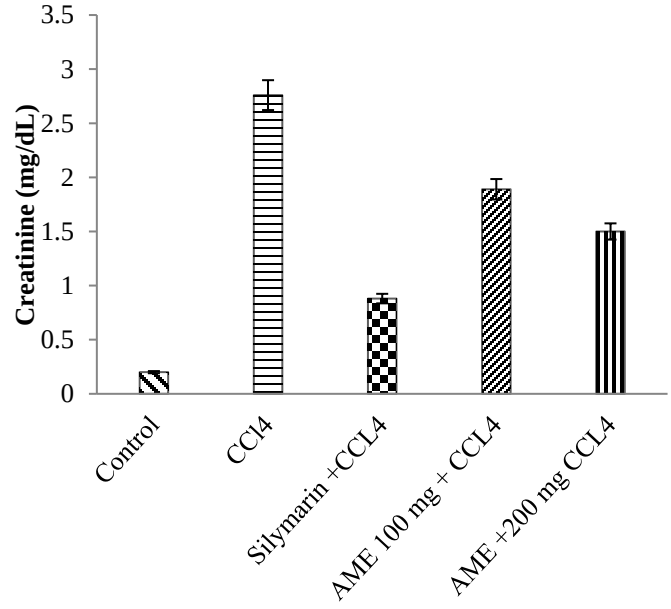
One of the most important aspects of evaluating renal function is the measurement of total creatinine. The kidneys are essential for blood filtration, helping to eliminate waste materials like creatinine. A higher-than-normal blood creatinine level may be a sign of compromised renal function. As the tiny standard deviation suggests, Group 1 is the control group, with a mean creatinine level that is quite low and has limited variability. Depending on the situation, people with reduced muscle mass or maybe 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 shown 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 [32-33].

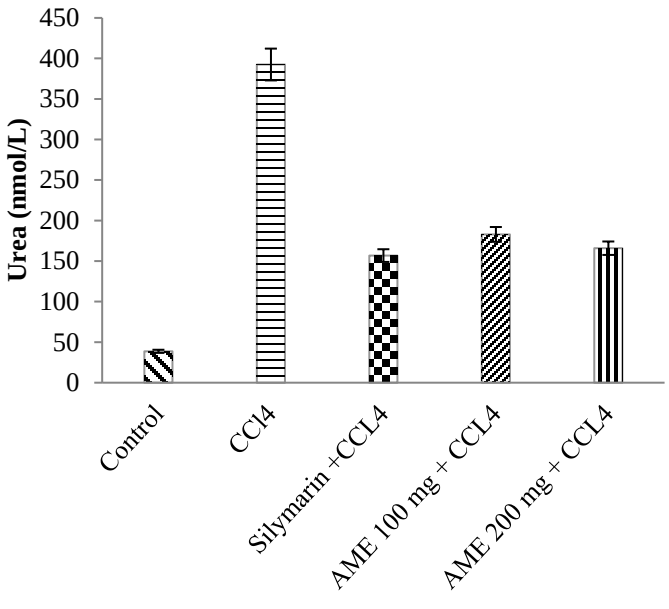
Table 1: Biochemical Parameters for Nephrotoxicity

Groups Name	Dose (mg/kg)	Estimation of Blood							
		Creatinine (mg/dL)	%	Urea (nmol/L)	%	Uric acid (mg/dL)	%	Total Protein (gm/L)	%
Control	1 mL	0.2±0.04	-	38.7±5.30	-	1.95±0.10	-	123.8±2.40	-
CCl_4	1.25 mL/kg	2.76±1.20	-	392.50±10.08	-	8.2±0.11	-	209.5±5.35	-
Silymarin	10	0.88±0.05	68	156.8±5.10	60	2.99±0.03	63	139.8±2.10	35
AME	100	1.89±0.58	32	182.9±9.05	53	5.99±1.01	26	197.8±6.41	4
AME	200	1.31±0.85	57	165.9±7.79	52	5.41±0.80	34	187.8±2.06	9

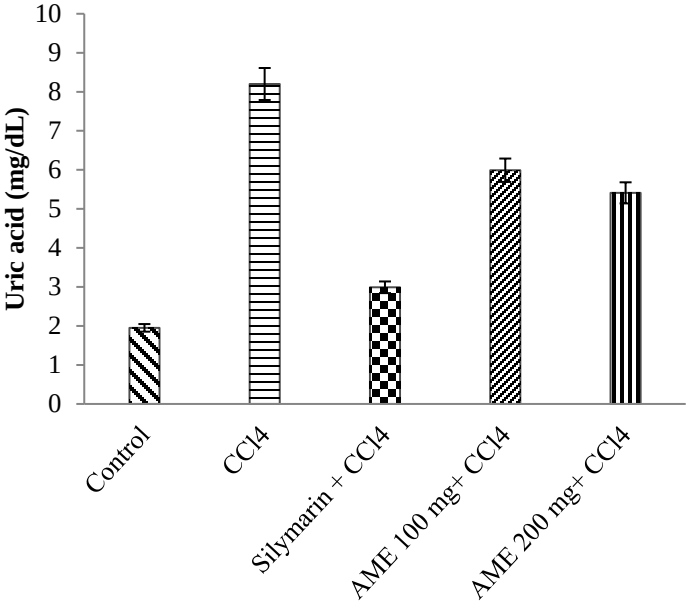
Values expressed as mean ± SEM of n = 6. $p<0.05$; ANOVA, followed by Dunnett's multiple comparison test % Represents % of change respect to CCl_4 group



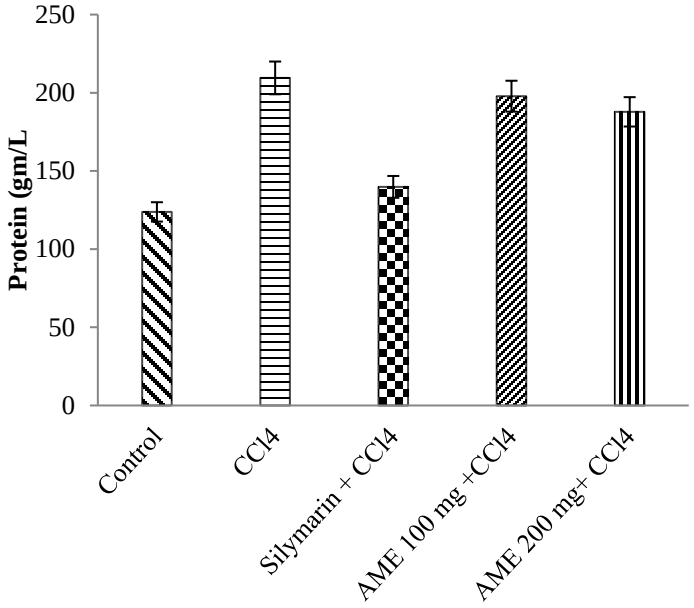
a. Level of Creatinine



b. Level of Urea



c. Level of Uric acid



d. Level of Protein

Figure 1: Biochemical Parameters (Creatinine, Urea, Uric Acid, Protein) for Nephrotoxicity

Biochemical Parameters for Hepatotoxicity

Damage to the liver impairs the operation of the hepatocyte transport system, leading to plasma membrane leakage, elevated blood enzyme levels, and tuberculosis. The liver typically eliminates bile's ALP, TB, SGOT, and SGPT enzymes. Hepatotoxins cause abnormalities in the liver's bile excretion pathway, raising blood enzyme levels [34]. 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 mostly present in the liver is 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

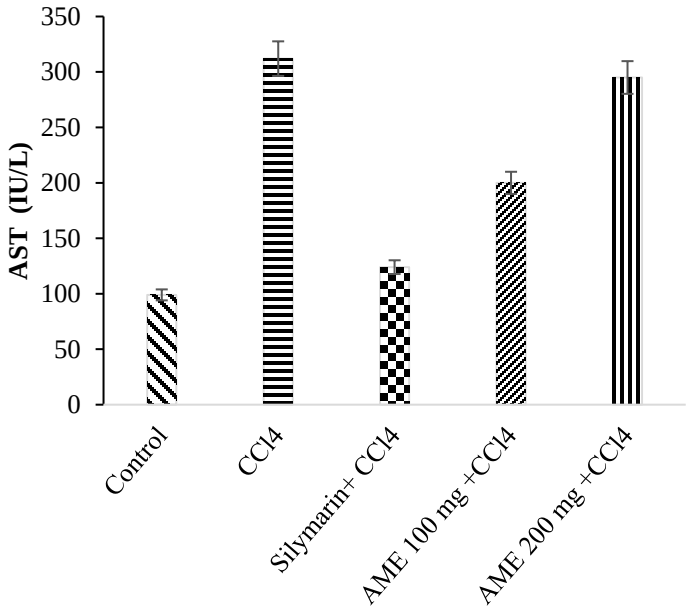
enzyme ALP. Elevated ALP readings may indicate bile system obstruction or liver or bone disease. The reference range is typically 30 to 120 IU/L. The liver produces the protein known as albumin, which is an essential component of blood plasma. Low total albumin levels can indicate liver disease, renal failure, malnourishment, or other conditions. The usual reference range is 3.4–5.4 g/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 shown 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 g/dl. Viral infections, heavy drug use, alcohol misuse, and a variety of hazardous substances can all result in liver damage. According to Bouhrim M *et al.*, the Hepatotoxic experimental model of damage caused by CCl_4 shares many physiological and pathological traits with human hepatotoxic liver injury. Geum-Hwa Lee et al. state that *CCl_4 damages the liver through lipid peroxidation, oxidative stress,* and inflammation. The CYP2E1 enzyme breaks down CCl_4 in the liver to produce the hazardous reactive trichloromethyl and trichloromethyl peroxide radicals [35-36]. These reactive radicals also attach to unsaturated fatty acids in the membranes of mitochondria, endoplasmic reticulum, and hepatocytes. This results in a chain of lipid peroxidation processes that damages and eventually kills intracellular structures and hepatocytes [37-38].

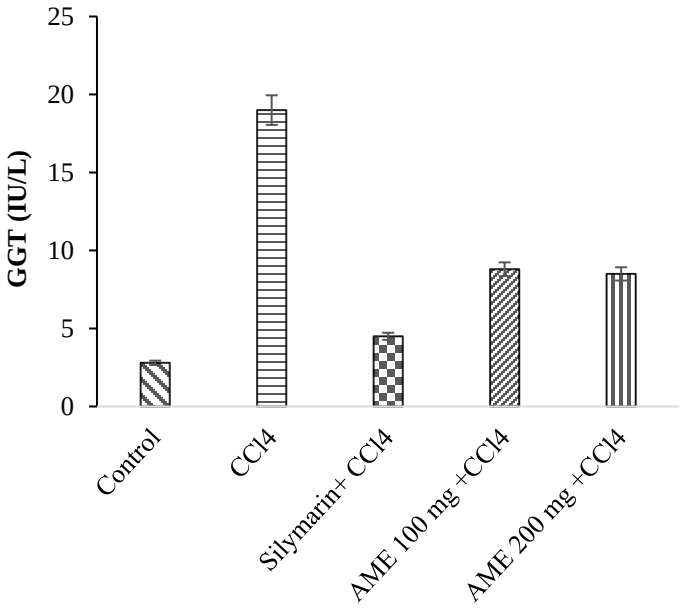
Table 2: Biochemical Parameters for Hepatotoxicity

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	97.27±3.25	-	28.18±2.45	-	2.5±3.55	-	61.9±2.11	-	0.35±2.55	-
CCl_4	1.25 ml/kg	310.01±7.50	-	172.48±5.88	-	18.6±4.44	-	365.56±3.89	-	2.42±1.11	-
Silymarin	10	125.9±1.52	59	64.07±4.54	62	4.68±6.78	77	114.79±1.44	68	0.77±1.87	67
AME	100	201.92±2.50	35	100.37±7.85	41	8.98±5.35	55	274.22±7.22	24	1.78±7.20	27
AME	200	296.92±3.45	45	128.22±6.66	25	8.57±3.88	52	230.69±6.88	36	2.1±4.88	16

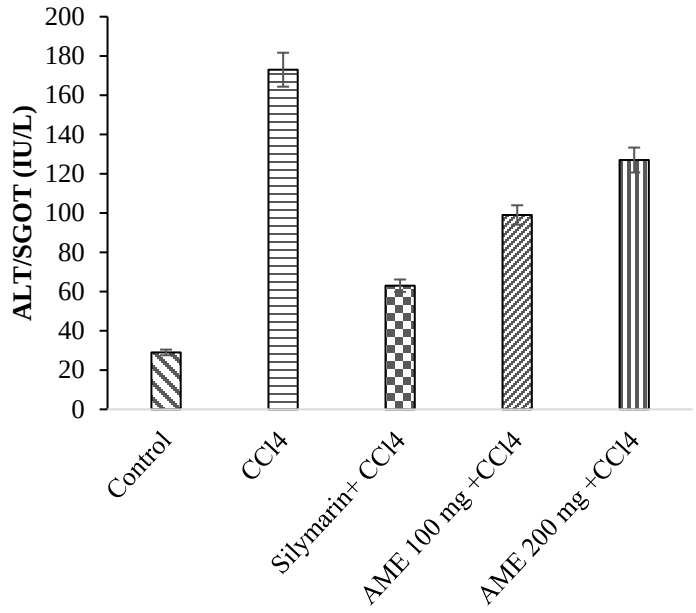
Values expressed as mean ± SEM of n = 6. $p<0.05$; ANOVA, followed by Dunnett's multiple comparison test AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyltranspeptidase; ALP: alkaline phosphatase.



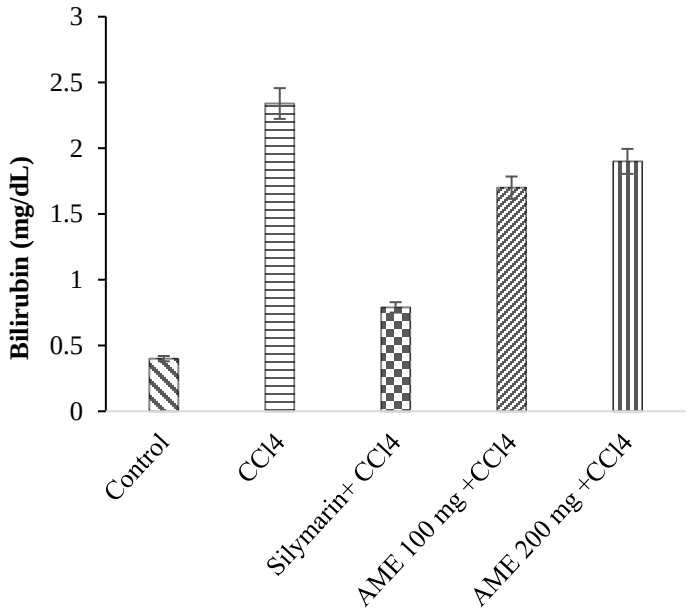
a. Level of AST



b. Level of GGT



c. Level of AST/SGOT



d. Level of Bilirubin

Figure 2: Biochemical Parameters (AST, ALT/SGOT,GGT, ALP/SGPT, Bilirubin) for Hepatotoxicity

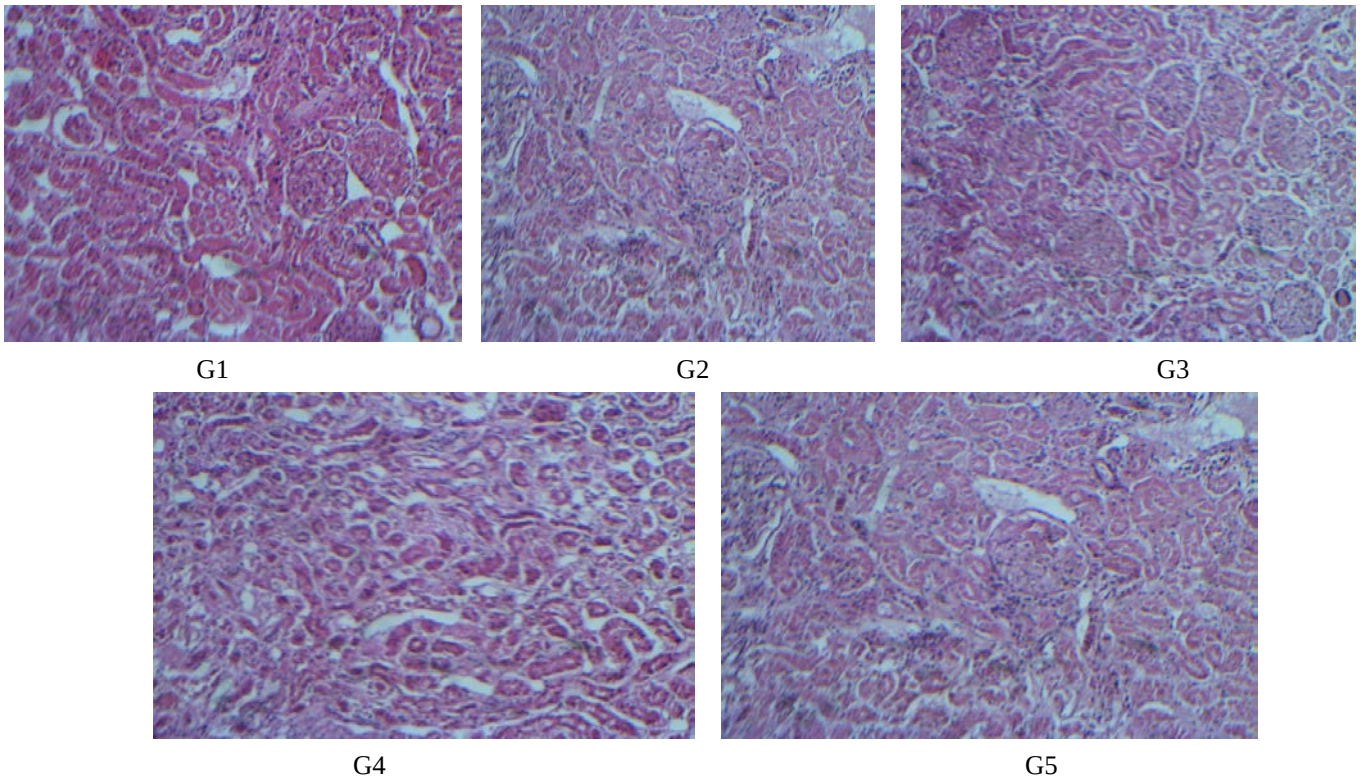
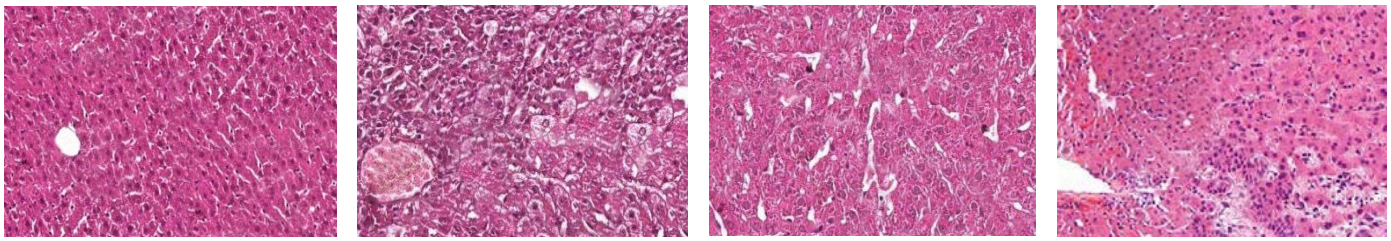


Figure 3: Histopathological Study of Kidney

HISTOPATHOLOGICAL EVALUATION

The histopathological studies demonstrate how efficient the drug is as a preventative measure. Under a microscope, the renal corpuscle and cortexes of the control kidney appeared normal. The glomerulus was surrounded by a thin renal or Bowman's space in these spherical, tight structures. On the other hand, rats on a high-casein diet displayed changes in their kidneys that were both cortical and modularly. For example, rats fed the diet left the large Bowman's gap in the experimental animals, and the glomerulus shrank. Additionally, rats displayed partial thickening of the capsule, localized tubular epithelial cell degeneration, and the emergence of tubular casts in the ducts, Henle loop, cortical tubules, and modularly tubules.

Normal kidney cells (G1); abnormal glomeruli reduced in size with almost no Bowman space; degenerated and convoluted necrotic tubules; abnormal medulla structure with inflammatory infiltrate; abnormal calyx structure blocked by hyaline and granulated materials (B) Rat kidney cells treated with CCl4. (G3) Kidney cells treated with CCl4 and silymarin exhibit disruption of the glomerulus architecture, decreasing size, decreased Bowman space, degeneration, and necrosis in the tubules and glomerulus, as well as cortical congestion, hyaline material accumulation, and inflammatory infiltration. (G4) Rats' kidney cells treated with CCl4 and 200 mg/kg of Aegle Marmelos total extract showed nearly normal calyxes with a mild presence of hyaline material, mild degeneration in a small number of tubule cells, and a few tubules obstructed by hyaline material and collagen fiber deposition. (A–C) or 20x, 200 µm (D) magnification.



G1 G2 G3 G4

Figure 4: Histopathological Study of Liver

(G1) Normal Liver Cells; (G2) Following the administration of CCl4, the liver cells showed multiple necrotic patches, notable hepatocyte vacuolization, fatty modifications, disruption of the parenchymal architecture, deposition of collagen fibers, a decrease in glycogen content, dilation of the sinusoids, congestion of the central venous circulation, and infiltration of inflammation. After exposure to CCl_4 and silymarin (G3), rat liver cells show signs of moderate necrosis, hepatocyte vacuolization, portal triage, and tiny nuclei aggregating toward the portal vein endothelium. (G4) Rat liver cells treated with 200 mg/kg body weight of Aegle Marmelos and CCl_4. Whole extract with no central venous congestion, several necrotic zones, fatty changes, and hepatocyte vacuolization. Magnification: 100 µm, 20x. Rats treated with *Bael* 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 by Bael. A small number of hepatocytes in the immediate area of the intralobular vein show almost little damage. The endothelium lining is smooth, save for one or two areas. Hepatocytes seem normal; many cells have more vacuoles in their cytoplasm. The findings of the biochemical parameters are confirmed by the histological investigation.

CONCLUSION

The study's conclusion may outline the key findings and implications of the investigation into the hepatoprotective and renoprotective effects of Bael (Fruit) on carbon tetrachloride-induced toxicity in rats. Two distinct dosages of Bael's Ethanolic extract (100 and 200 mg/kg/day) were compared with 100 mg/kg of silymarin. The rats were given these dosages for five days, and one hour after treatment on the third and fourth days, CCl4 (50 mg/kg i.p.) was given. The animals were slaughtered 48 hours following their final CCl4 dose. Biochemical blood parameters and histopathologic changes in the kidney and liver were evaluated. The ethanolic extract, at 200 mg/kg/day, was more effective in reducing the elevated blood levels of ALT, AST, ALP, urea, and creatinine.

According to the histo-pathological assessment, Bael lessened the CCl4-induced hepatic and renal necrosis. Our findings suggest that the antioxidant properties of Bael could contribute to the claimed protective advantages. It strengthens cells' defense mechanisms against oxidative stress, reducing oxidative damage to the liver and kidneys. The findings of this study have implications for the health of the kidneys and liver, particularly about damage brought on by pollutants. Bael extract may be therapeutically beneficial in protecting these vital organs from harmful attacks. It is strongly advised that the extract fractions and constituents be investigated further. In conclusion, our study provides evidence for the reno-protective and hepatoprotective qualities of Bael fruit in a rat model of carbon tetrachloride contamination.

The implications for future research include a strong recommendation to investigate further the specific fractions and

constituents of the Bael extract that confer these protective effects. Understanding these components at a molecular level could lead to the development of more targeted and effective therapeutic agents. For clinical practice, our findings suggest that Bael extract could be considered as a complementary treatment for liver and kidney protection, especially in conditions where these organs are at risk of damage due to exposure to toxic substances. This study provides a foundation for future clinical trials to validate Bael's efficacy and safety in human populations, potentially leading to new preventive and therapeutic healthcare applications.

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FINANCIAL ASSISTANCE
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. Deepak Garg 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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