

Evaluation of Hepatoprotective Activity of Extract of *Cyamopsis Tetragonoloba*

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ABSTRACT:

Background and Objectives: Liver problems are a global health issue, and scientists are still searching for natural hepatoprotectants. Guar, a medicinal plant rich in phenolic compounds, flavonoids, and antioxidants, may protect the liver. The study examined the hepatoprotective effects of *Cyamopsis tetragonoloba* methanolic extract against CCl₄-induced hepatotoxicity in rats. Hepatoprotective and antioxidant properties of *C. tetragonoloba* methanolic extracts have been found.

Methods: The hepatoprotective activity was assessed in 30 Wistar rats using a model of liver injury caused by CCl₄. Six animals were split into five groups: one that served as a control, one that was poisonous (CCl₄), one that served as a standard (silymarin, 100 mg/kg), and two that were treated with methanolic extract (100 and 200 mg/kg). The course of treatment lasted for fourteen days. We evaluated serum biochemical parameters such as SGOT (AST), SGPT (ALT), alkaline phosphatase (ALP), total bilirubin, and total protein. The hepatic architecture and cellular damage were evaluated through histopathological investigation of liver samples.

Results: There was a substantial increase in serum SGOT (168.4 ± 8.6 U/L), SGPT (142.7 ± 7.9 U/L), ALP (286.5 ± 12.4 U/L), and total bilirubin (2.84 ± 0.21 mg/dL) when CCl₄ was administered compared to the normal control group ($p < 0.001$). The results showed that total bilirubin (1.18 ± 0.12 mg/dL), SGOT (92.6 ± 5.2 U/L), SGPT (78.4 ± 4.8 U/L), ALP (178.3 ± 10.2 U/L), and total methanolic extract from *C. tetragonoloba* (200 mg/kg) were all significantly reduced, with an increase in total protein levels to 6.72 ± 0.31 g/dL. There was a level of hepatoprotective activity similar to silymarin. The histopathological results showed that the groups treated with the extract had significantly restored hepatic architecture, less necrosis, and less inflammatory cell infiltration. These results corroborate previous reports of hepatoprotective effects of *C. tetragonoloba* methanolic extracts.

Conclusion: The methanolic extract of *Cyamopsis tetragonoloba* showed considerable hepatoprotective action against CCl₄-induced liver damage, as shown by normalised biochemical indicators and improved liver histology. The activity may be due to antioxidant phytoconstituents such phenolics and flavonoids. *C. tetragonoloba* may be a natural hepatoprotectant, however its active ingredients need to be isolated and characterised.

Keywords: *Cyamopsis tetragonoloba*; Hepatoprotective activity; Carbon tetrachloride; Liver enzymes; Antioxidant activity; Silymarin; Histopathology.

INTRODUCTION:

The liver is a crucial organ in the human body, integral to metabolism, detoxification, nutrient storage, and the creation of important macromolecules. Contact with pharmaceuticals, environmental pollutants, ethanol, and pathogens can cause hepatic damage, leading to compromised liver functionality and the emergence of several hepatic ailments ¹. Notwithstanding progress in contemporary medicine, the treatment of liver ailments continues to be difficult owing to the scarce availability of efficacious hepatoprotective medications and their related side effects. As a result, there is an increasing fascination with discovering natural substances that possess hepatoprotective properties ^{2,3}.

Herbal remedies have been extensively utilised in conventional medical practices for the prophylaxis and management of hepatic disorders. Phytochemicals sourced from plants, including flavonoids, phenolic compounds, alkaloids, and saponins, have antioxidant and free radical scavenging capabilities that aid in

liver safeguarding. These organic substances aid in diminishing oxidative stress, obstructing lipid peroxidation, and facilitating the regeneration of impaired hepatocytes ^{4,5}.

Cyamopsis tetragonoloba (L.) Taub., referred to as guar, is a member of the Fabaceae family and is extensively grown in India and various tropical areas. The plant is acknowledged for its nutritional significance and has an array of bioactive components such as flavonoids, phenolic substances, proteins, and polysaccharides ⁶. Prior research has indicated the antioxidant, anti-inflammatory, antidiabetic, and antibacterial properties of *C. tetragonoloba*, implying its possible medicinal uses ⁷.

Given the extensive phytochemical composition and biological properties of the plant, this investigation was conducted to assess the hepatoprotective effects of the methanolic extract of *Cyamopsis tetragonoloba* against carbon tetrachloride (CCl₄)-induced liver damage in experimental rats. The research evaluated serum biochemical indicators and histological alterations to ascertain the extract's protective influence on hepatic function and tissue structure ⁹.

MATERIAL AND METHODS:

Plant Material and Extraction:

Fresh fruits of *Cyamopsis tetragonoloba* were collected and authenticated. The fruits were washed, shade-dried, and powdered using a mechanical grinder. The powdered material was extracted with methanol in a Soxhlet apparatus for 24 hours. The obtained extract was filtered and concentrated under reduced pressure using a rotary evaporator at controlled temperature. The concentrated extract was dried, the percentage yield was calculated, and the extract was stored in an airtight container at 4°C until further use.

Experimental Animals:

A group of healthy adult male and female Wistar albino rats, ranging in weight from 150 to 200 g, were selected for the experiment after a week of acclimatisation to laboratory settings. The animals were kept in polypropylene cages in a controlled environment with a 12-hour light/dark cycle, 25 ± 2°C temperature, 55 ± 5% relative humidity, and an unlimited supply of water and standard pellet food. All operations were conducted in compliance with CPCSEA guidelines for the care and use of laboratory animals, and the experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) ^{10,11}.

Acute Toxicity Study:

The methanolic *Cyamopsis tetragonoloba* extract was subjected to an acute oral toxicity analysis in accordance with OECD guideline 423 (Acute Toxic Class Method). After oral administration of the extract at varying doses, the animals were monitored for toxicity, behavioural changes, and death for a duration of 14 days. Up to a dosage of 2000 mg/kg body weight, the extract did not cause any death or other hazardous effects, hence it was safe to use. The hepatoprotective study's doses were chosen at 100 mg/kg and 200 mg/kg body weight based on these results ¹².

Experimental Design:

Five groups of six Wistar albino rats were randomly selected from 30. Group I, the control, received normal saline throughout the research. Group II, the toxic control, was given CCl₄ to induce hepatotoxicity. In Group III, silymarin (100 mg/kg body weight) was given with CCl₄, whereas Groups IV and V got *Cyamopsis tetragonoloba* methanolic extract (100 mg/kg and 200 mg/kg body weight, respectively) with CCl₄. To induce hepatotoxicity, CCl₄ was combined with liquid paraffin (1:1, v/v) and given intraperitoneally at 1 mL/kg body weight on alternate days for 14 days. The usual medication and plant extract treatments were taken orally daily during the experiment. At the end of the trial, blood and liver samples were taken for biochemical and histological hepatoprotective activity testing ¹³.

Biochemical Analysis:

The experimental animals underwent retro-orbital puncture under light anaesthesia to collect blood samples at the conclusion of the treatment period. After letting the samples clot, they were spun in a centrifuge at 3000 rpm for 10 minutes to extract serum that was clear. Using commercially available standard diagnostic kits, the separated serum was used to estimate liver function parameters such as serum glutamate oxaloacetate transaminase (SGOT/AST), serum glutamate pyruvate transaminase (SGPT/ALT), alkaline phosphatase (ALP), total bilirubin, and total protein. A methanolic extract of *Cyamopsis*

tetragonoloba was tested for its hepatoprotective effects and the severity of liver injury using these biochemical markers ¹⁴.

Histopathological Examination:

After blood was collected, the animals were killed and their livers were painstakingly removed. Then, to eliminate any blood stains, the tissues were rinsed with normal saline and preserved in 10% neutral buffered formalin. The paraffin-embedded tissues were sectioned into 5 µm thick pieces using a microtome after undergoing conventional histological processing on the preserved tissues. Haematoxylin and eosin (H&E) staining allowed for light microscopy examination of the sections, which revealed histological alterations including fatty degeneration, hepatocellular necrosis, inflammatory cell infiltration, and normalisation of the liver's architecture. A hepatoprotective effect of *Cyamopsis tetragonoloba* methanolic extract was evaluated using the data collected ¹⁵.

Statistical Analysis:

Data were expressed as mean ± standard deviation (SD) for six animals in each group. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A value of $p < 0.05$ was considered statistically significant.

RESULTS:

Effect of *Cyamopsis tetragonoloba* Extract on Serum Biochemical Parameters:

The normal control group showed reduced total protein levels and increased blood levels of SGOT, SGPT, ALP, and total bilirubin, all of which indicated that carbon tetrachloride (CCl₄) administration caused substantial liver damage. These biochemical markers were markedly and dose-dependently brought back to normal after treatment with the *Cyamopsis tetragonoloba* methanolic extract. Greater hepatoprotective effect was observed at the higher dose (200 mg/kg), with findings that were comparable to those of the standard medication silymarin.

Table 1: Effect of *Cyamopsis tetragonoloba* Extract on Liver Function Parameters

Group	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Total Bilirubin (mg/dL)	Total Protein (g/dL)
Normal Control	62.4 ± 4.3	48.6 ± 3.7	124.8 ± 8.5	0.68 ± 0.08	7.12 ± 0.34
Toxic Control (CCl ₄)	168.4 ± 8.6	142.7 ± 7.9	286.5 ± 12.4	2.84 ± 0.21	4.28 ± 0.26
Silymarin (100 mg/kg)	76.8 ± 5.1	58.9 ± 4.3	142.6 ± 9.2	0.82 ± 0.09	6.98 ± 0.32
Extract (100 mg/kg)	118.3 ± 6.5	97.6 ± 5.8	214.7 ± 11.5	1.76 ± 0.15	5.84 ± 0.29
Extract (200 mg/kg)	92.6 ± 5.2	78.4 ± 4.8	178.3 ± 10.2	1.18 ± 0.12	6.72 ± 0.31

Severe hepatic damage was indicated by the toxic control group's elevated liver enzyme levels and bilirubin concentration as compared to the normal control group. *Cyamopsis tetragonoloba* extract treatment improved total protein content and dramatically decreased SGOT, SGPT, ALP, and bilirubin levels. When compared to the 100 mg/kg dose, the 200 mg/kg dose was more protective and came close to silymarin's efficacy.

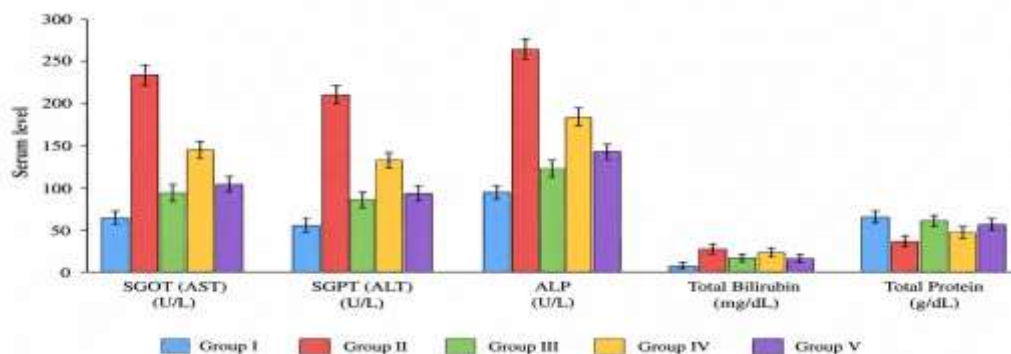


Figure 1: Effect of Cyamopsis tetragonoloba Extract on Serum Liver Biomarkers

Figure 1 shows how different experimental groups' serum SGOT, SGPT, ALP, total bilirubin, and total protein levels changed. The methanolic extract lowered these indicators in a dose-dependent manner, while CCl₄ injection dramatically raised liver damage markers. Similar to the usual medicine, the larger dose of the extract restored liver function substantially.

Effect on Relative Liver Weight:

Because of inflammation and cellular degradation, CCl₄ intoxication greatly increased liver weight. By restoring normal liver morphology and decreasing liver size, the extract proved to be an effective treatment.

Table 2: Effect on Relative Liver Weight

Group	Relative Liver Weight (%)
Normal Control	3.18 ± 0.14
Toxic Control (CCl ₄)	5.46 ± 0.22
Silymarin (100 mg/kg)	3.42 ± 0.18
Extract (100 mg/kg)	4.28 ± 0.19
Extract (200 mg/kg)	3.68 ± 0.16

When contrasted with healthy animals, the toxic control group's relative liver weight was much higher. The dose-dependent reduction of liver enlargement after administering Cyamopsis tetragonoloba extract suggests protection against CCl₄-induced hepatic damage.

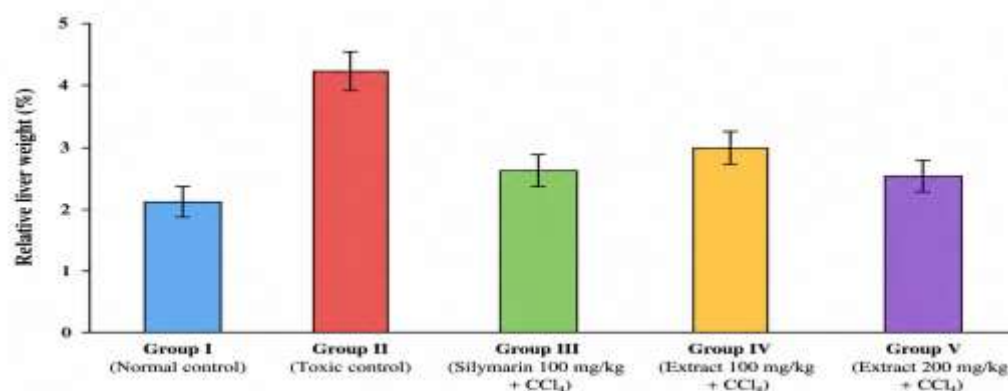


Figure 2: Effect of Cyamopsis tetragonoloba Extract on Relative Liver Weight

The proportional weight of the animals' livers in the experiment is shown in Figure 2. The liver weight of rats given CCl₄ increased significantly, while rats given extract showed a considerable decrease, especially at the 200 mg/kg dose.

Histopathological Evaluation:

Histopathological analysis showed extensive hepatic necrosis, lipid alterations, and inflammatory infiltration in the livers of the toxic control group. Hepatocyte regeneration and recovery were significantly enhanced with larger doses of the extract, demonstrating a dose-dependent improvement in liver architecture. Liver sections were examined under a microscope to confirm the biochemical results.

Table 3: Histopathological Observations of Liver Tissue

Group	Histopathological Findings
Normal Control	Normal hepatic architecture with intact hepatocytes and central vein
Toxic Control (CCl ₄)	Severe hepatocellular necrosis, fatty degeneration, inflammatory infiltration, and sinusoidal congestion
Silymarin (100 mg/kg)	Near-normal hepatic architecture with minimal cellular degeneration
Extract (100 mg/kg)	Moderate reduction in necrosis and inflammation with partial restoration of hepatocytes
Extract (200 mg/kg)	Marked restoration of liver architecture with mild inflammatory changes

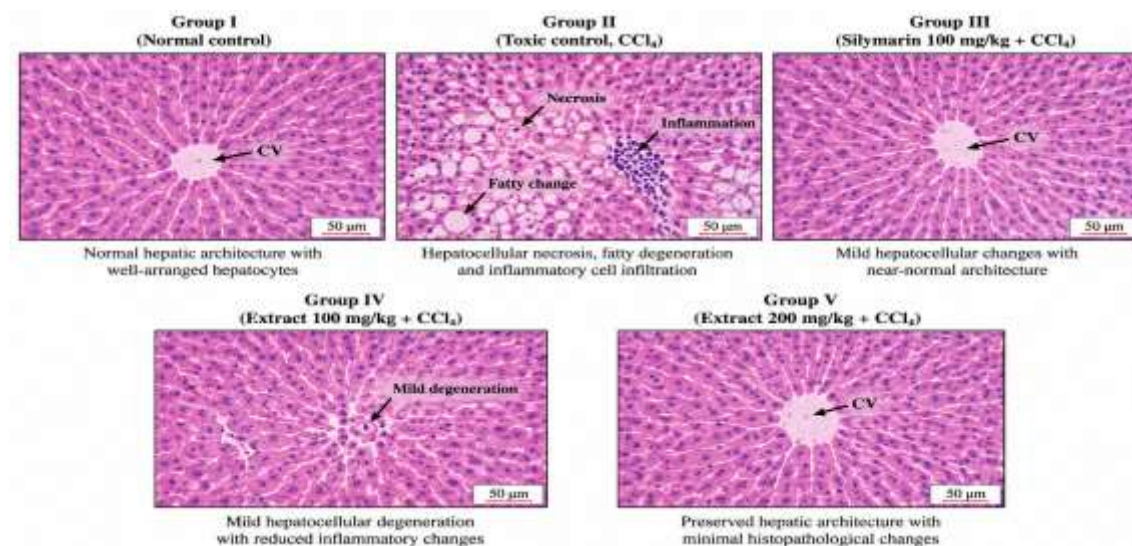


Figure 3: Histopathological Sections of Liver Tissue

Photomicrographs of liver slices from several experimental groups are displayed in Figure 3. The hepatic architecture was found to be intact in normal animals, but rats that were treated with CCl₄ showed signs of severe necrosis and fatty degeneration. Groups treated with the extract showed a gradual improvement in hepatocyte structure and a decrease in inflammatory alterations, proving that *Cyamopsis tetragonoloba* had hepatoprotective effects.

Percentage Hepatoprotection:

The reduction in SGOT levels in comparison to the toxic control group was used as the basis for the calculation of the percentage of hepatoprotection measured.

Table 4: Percentage Hepatoprotection

Group	Hepatoprotection (%)
Silymarin (100 mg/kg)	86.4
Extract (100 mg/kg)	47.3

Extract (200 mg/kg)	71.5
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Cyamopsis tetragonoloba methanolic extract showed strong hepatoprotective effects. Strong liver-protective potential was shown by the 71.5% hepatoprotection at the higher dose of 200 mg/kg, which was close to the 86.4% protection attained by the standard medication silymarin.

DISCUSSION:

This study tested if the methanolic extract of *Cyamopsis tetragonoloba* could protect experimental rats' livers from CCl₄-induced damage. An recognised hepatotoxic agent, carbon tetrachloride damages the liver by causing lipid peroxidation, cellular membrane destruction, and hepatocellular necrosis through the production of free radicals and reactive oxygen species. The current study confirmed the development of hepatic damage by showing that blood levels of SGOT, SGPT, ALP, and total bilirubin were considerably elevated after CCl₄ injection, whereas total protein levels were decreased ^{16,17}.

Cyamopsis tetragonoloba methanolic extract treatment markedly and dose-dependently returned these abnormal metabolic parameters to normal levels. Lower levels of bilirubin and serum transaminases suggest that hepatocyte membranes have stabilised and that intracellular enzyme leakage into the bloodstream has been prevented. Total protein levels have gone up, which is more evidence of better liver synthesis function and the ability to repair damaged liver tissue ¹⁸.

In addition to biochemical evidence, histopathological analysis corroborated the results. Liver architecture was significantly improved in the extract-treated groups, with reduced inflammation and necrosis, in contrast to the toxic control group that displayed severe hepatic degeneration, necrosis, inflammatory cell infiltration, and sinusoidal congestion. Greater hepatoprotective efficacy and effects equal to the standard medication silymarin were achieved by the higher dose of the extract (200 mg/kg) ^{19, 20}.

The presence of bioactive phytoconstituents such flavonoids, phenolic compounds, and other antioxidant molecules may explain why *Cyamopsis tetragonoloba* has a hepatoprotective effect. It is well-known that these chemicals can protect hepatocytes from oxidative damage, prevent lipid peroxidation, and neutralise free radicals. The considerable improvement in biochemical and histological indicators indicates that the extract may have hepatoprotective effects through antioxidant mechanisms ^{21, 22}. The results show that *Cyamopsis tetragonoloba*'s methanolic extract protects the liver from CCl₄-induced damage, lending credence to its long-standing use as a remedy for liver problems ²³.

CONCLUSION:

This study showed that experimental rats were protected from carbon tetrachloride-induced liver damage by using a methanolic extract of *Cyamopsis tetragonoloba*. The blood liver enzyme levels, bilirubin concentration, total protein content, and hepatic architecture were all returned to normal after treatment with the extract. The hepatoprotective effect was dose-dependent, appearing to be on par with the gold standard medication silymarin at a dosage of 200 mg/kg. It is possible that the antioxidant phytoconstituents, including flavonoids and phenolics, are responsible for the observed protective effect. According to these results, *Cyamopsis tetragonoloba* could be a powerful natural hepatoprotective agent, so it's important to figure out what makes it tick.

Funding:

Not applicable.

Conflict of interest:

The authors declare that they have no conflict of interest.

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