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Research Article

Study the Effect of Prosopis cineraria Seeds on Paracetamol-Induced Hepatotoxicity in Albino Rats

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Abstract:

Background: Drug-induced liver injury remains a major health concern, with paracetamol (acetaminophen) being one of the leading causes of hepatotoxicity when consumed in overdose. The present study investigates the hepatoprotective potential of Prosopis Cineraria seed extract against paracetamol-induced liver damage in albino rats.

Methods: Adult Wistar albino rats were divided into five groups (n=6). Group I served as the normal control, receiving only the vehicle. Group II received paracetamol (2 g/kg body weight) to induce hepatotoxicity. Group III was treated with standard drug silymarin (100 mg/kg) following paracetamol administration. Groups IV and V were pretreated with ethanolic extract of Prosopis Cineraria seeds at doses of 200 mg/kg and 400 mg/kg, respectively, before paracetamol administration. Serum levels of hepatic enzymes (ALT, AST, ALP), total bilirubin, and liver histopathology were evaluated to assess hepatoprotective activity.

Results: Paracetamol administration significantly elevated liver enzymes and total bilirubin levels compared to the control group ($p < 0.001$), indicating hepatic damage. Pretreatment with Prosopis Cineraria extract resulted in a dose-dependent reduction in these markers, with the 400 mg/kg dose showing comparable effects to silymarin. Histopathological examination supported these findings, revealing reduced hepatic necrosis and inflammation in extract-treated groups.

Conclusion: The ethanolic extract of Prosopis Cineraria seeds exhibited significant hepatoprotective activity against paracetamol-induced liver injury in albino rats. These findings suggest that Prosopis Cineraria may serve as a potential natural therapeutic agent for managing liver disorders.

Keywords: Prosopis cineraria, Hepatotoxicity, Paracetamol, Hepatoprotective effect, Albino rats, Ethanolic extract, Liver enzymes, Silymarin, Histopathology, Herbal hepatoprotective agent.

Introduction

The liver is a vital organ responsible for various physiological functions, including metabolism, detoxification, bile secretion, and storage of essential nutrients. Due to its central role in the biotransformation and elimination of drugs and toxins, the liver is highly vulnerable to injury caused by xenobiotics. Among drug-induced liver injuries, paracetamol (acetaminophen) toxicity is one of the most commonly reported and clinically relevant causes of acute liver failure worldwide. Though paracetamol is safe at therapeutic doses, its overdose can result in

the accumulation of a toxic metabolite, N-acetyl-p-benzoquinone imine (NAPQI). This reactive intermediate depletes hepatic glutathione reserves and triggers oxidative stress, mitochondrial dysfunction, and necrosis of hepatocytes. Therefore, paracetamol-induced hepatotoxicity serves as a widely accepted experimental model for evaluating the hepatoprotective efficacy of potential therapeutic agents [1,2,3].

In recent years, there has been a growing interest in the use of medicinal plants and their bioactive constituents as alternative approaches for the prevention and treatment of liver diseases. Herbal medicines are considered safer and more cost-effective compared to synthetic drugs. They are rich sources of phytochemicals such as flavonoids, tannins, alkaloids, saponins, and phenolic compounds, many of which exhibit strong antioxidant and anti-inflammatory properties that can protect liver cells against toxic damage. The search for effective hepatoprotective agents from natural sources continues to be an important area of research, especially in the context of traditional medicine systems like Ayurveda [4,5].

Prosopis Cineraria, commonly known as Khejri, belongs to the family Fabaceae and is a well-known medicinal tree native to arid regions of India, especially Rajasthan. Traditionally, different parts of this plant have been used in the treatment of ailments such as asthma, bronchitis, dysentery, skin disorders, and inflammatory conditions. Although the therapeutic potential of Prosopis Cineraria has been explored in various studies for its antioxidant, antimicrobial, anti-inflammatory, and antidiabetic properties, scientific data supporting its hepatoprotective effects are limited, particularly with regard to its seeds. The seeds are believed to contain beneficial phytochemicals that may offer protection against oxidative damage and liver injury [6,7,8].

The present study aims to investigate the hepatoprotective effect of ethanolic extract of Prosopis Cineraria seeds against paracetamol-induced hepatotoxicity in albino rats. Ethanolic extraction is preferred due to its efficiency in extracting a broad spectrum of phytoconstituents. The study compares the extract's efficacy at two different doses with that of silymarin, a standard hepatoprotective agent derived from *Silybum marianum*. Biochemical parameters such as serum ALT, AST, ALP, and total bilirubin levels will be assessed to determine the extent of liver injury and recovery. Histopathological examination of liver tissues will also be performed to support the biochemical findings and confirm the protective effect at the cellular level [9,10,11].

This research not only seeks to validate the traditional claims regarding Prosopis Cineraria but also aims to identify a promising natural hepatoprotective agent that could be further explored for clinical applications. The study contributes to the growing field of plant-based hepatoprotective drug development and supports the integration of herbal medicine into modern therapeutic approaches for liver disorders [12].

Material and Methods

Materials and Methodology

Plant Material and Extraction

The seeds of Prosopis Cineraria were collected from a local market in Jaipur and authenticated by the Department of Botany, University of Rajasthan. The seeds were shade-dried, coarsely powdered, and subjected to Soxhlet extraction using 90% ethanol. The extract was concentrated under reduced pressure, yielding a semisolid mass. The final yield of ethanolic extract was approximately 6.8% w/w. The extract was stored in airtight containers and used for experimental studies [13,14].

Animals

Healthy adult Wistar albino rats of either sex (150–200 g) were obtained from a licensed breeder. The animals were housed in standard laboratory conditions (25±2°C, 12-hour light/dark cycle, relative humidity 30–70%) and allowed free access to water and standard pellet diet. The study was approved by the Institutional

Animal Ethics Committee (IAEC) and conducted in accordance with CPCSEA guidelines [15.16].

Acute Toxicity Study

Acute toxicity was evaluated following OECD guideline 423. The ethanolic extract of Prosopis Cineraria was administered orally in increasing doses, and the animals were monitored for behavioral changes and mortality for 24 hours [17,18].

Paracetamol-Induced Hepatotoxicity Model

Animals were divided into four groups (n=6).

- Group I (normal control): received normal saline for 5 days.
- Group II (toxic control): received paracetamol (1000 mg/kg p.o.) on day 5.
- Group III (test group): received ethanolic extract of Prosopis Cineraria (400 mg/kg p.o.) for 5 days, followed by paracetamol on day 5.
- Group IV (standard group): received silymarin (50 mg/kg p.o.) for 5 days, followed by paracetamol on day 5.

Twenty-four hours after paracetamol administration, blood samples were collected via tail vein. Serum was separated for biochemical analysis of liver function markers, including SGOT, SGPT, SALP, and SOD using diagnostic kits [19,20].

Histopathological Examination

Rats were sacrificed, and liver tissues were collected and preserved in 10% formalin. Fixed tissues were processed, embedded in paraffin, sectioned (5–6 µm), and stained with hematoxylin and eosin (H&E). Sections were examined under a microscope for hepatic architecture and cellular integrity [21,22].

Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Statistical significance was determined using one-way ANOVA followed by Dunnett's and Tukey's post-hoc tests. A p-value <0.05 was considered statistically significant [23].

Result & Discussion:

Results of Acute Toxicity study:

There was no change in normal behavioural pattern of animals and no sign and symptoms of toxicity were observed during the observations which was done continuously for the first two hours and then observed up to twenty-four hours for mortality. The extracts were safe up to a maximum dose of 2000 mg/ kg body weight. The biological evaluation was carried out at doses 400 mg/kg body weight.

Results of preliminary phytochemical screening:

Table 1: Results of preliminary phytochemical screening

Sl. No.	Name of the Test	Observation	Conclusion
		Alcoholic Extract	
I.	**Tests for Steroids**		
	Salkowski reaction	+	Steroids were present in alcoholic extract.
	Liebermann		
	Burchard	+	
	Liberman's reaction	+	
II.	**Tests for Saponins**		Saponins were present in alcohol extracts.
	Foam test	+	
	Haemolytic test	+	
III.	**Tests for Tannins and Phenolic Compounds**		Tannins were present in alcoholic extracts whereas Phenolic
	Lead acetate test	+	
	5% Fe Cl_3 test	+	

	Bromine water test Acetic acid solution test Potassium dichromate Test	+ + +	compounds were present in alcoholic extract only.
V.	**Tests for Flavonoids** Shinoda test Lead acetate test Alkaline solution Ferric chloride test	 + + + +	Flavanoids were present extracts.
VI.	**Tests for Reducing Sugars** Fehling's test Benedict's test	 - -	Reducing sugars were not present in extract.

Thus, we can conclude from above observations that ethanolic extract contain steroids, saponins, tannins, phenolic compounds and flavonoids (Table No. 1)

Hepatoprotective activity

Paracetamol induced hepatoxicity:

Paracetamol at dose of 1000 mg/kg produced severe hepatotoxicity as indicated by sharp and significant (P<0.001) increase in SGOT, SGPT and SALP values in the control animals- 1348.00 ± 17.50, 585.00 ± 4.12, 257.50 ± 4.78 U/L respectively as compared to- 152.50 ± 1.75, 71.00 ± 1.08, 79.25 ± 1.10 U/L of normal animals.

SGOT:

All the extracts and silymarin were found to significantly (P<0.01) reduce the SGOT levels when compared with the control group. All the extracts EEPC 400 decrease in SGOT level which was similar (P<0.05) to that of standard. (Table No. 2 and Fig. No. 1)

SGPT:

EEPC 400 found to produce SGPT lowering effect similar (P<0.05) to the standard, where as EEPC 400 was better (P<0.01) than the standard. It was also found that the water extract produced better reduction of SGPT when compared to ethanolic extract. (Table No. 2 and Fig. No. 2)

SALP:

There was a significant (P<0.01) lowering of SALP levels by both the standard and the extracts when compared to the control group. EEPC 400 were found to produce SALP lowering effect similar to standard (P<0.01). (Table No. 2 and Fig. No. 3)

Table 2: Effect of extracts of Prosopis Cineraria seed extracts on biochemical parameters of liver in paracetamol induced hepatotoxicity in rats

Treatment	SGOT (U/L)	SGPT (U/L)	SALP (U/L)
Normal	152.50 ± 1.75	71.00 ± 1.08	79.25 ± 1.10
Control (Paracetamol)	1348.00 ± 17.50***†	585.00± 4.12***†	257.50 ± 4.78***†
Silymarin + Paracetamol	196.30 ± 1.75 **[a,]	78.50 ± 2.10**[a,]	126.80 ± 2.21**[a,]
EEPC 400 + Paracetamol	176.80 ± 3.56 **[a,*b]	48.50±5.17**[a, **b]	130.80 ± 1.49**[a, *b]

Values are the mean ±SEM, n=6, where, EEPC 400, indicates Ethanolic extract of Prosopis Cineraria. SGOT-serum glutamate oxalate transaminase, SGPT- Serum glutamate pyruvate transamsinase, SALP- Serum alkaline phosphatase, *Symbols represent statistical significance.** $P < 0.01$, *$P < 0.05$.'†'as control compared to normal 'a' as compared with paracetamol control and 'b' as compared with silymarin.

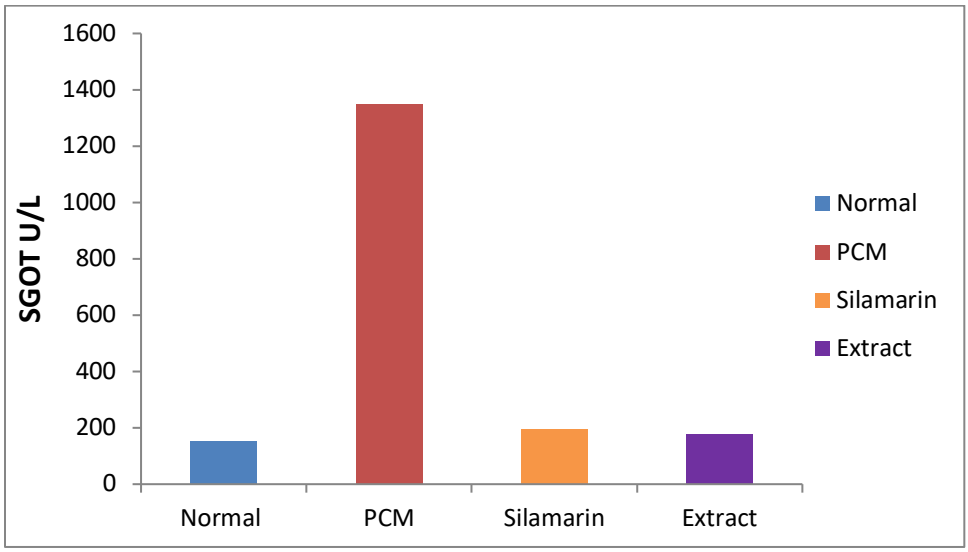


Figure 1: Effect of extracts of Prosopis Cineraria seed on SGOT levels of paracetamol intoxicated rats

Values are mean ±SEM, n=6, where, SGOT-serum glutamate oxalate transaminase EEPC 400 indicates Ethanolic Extarct of Prosopis Cineraria seed

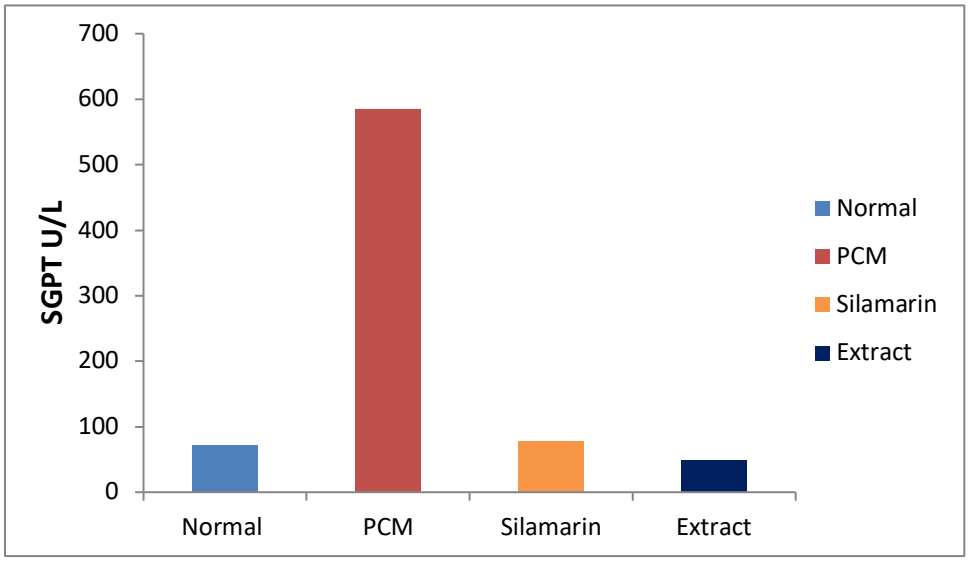


Figure 2: Effect of extracts of Prosopis Cineraria seed on SGPT levels of paracetamol intoxicated rats

Values are mean ±SEM, n=6, where, SGPT-Serum glutamate pyruvate Transaminase. EEPC 400 indicates Ethanolic Extarct of Prosopis Cineraria seed

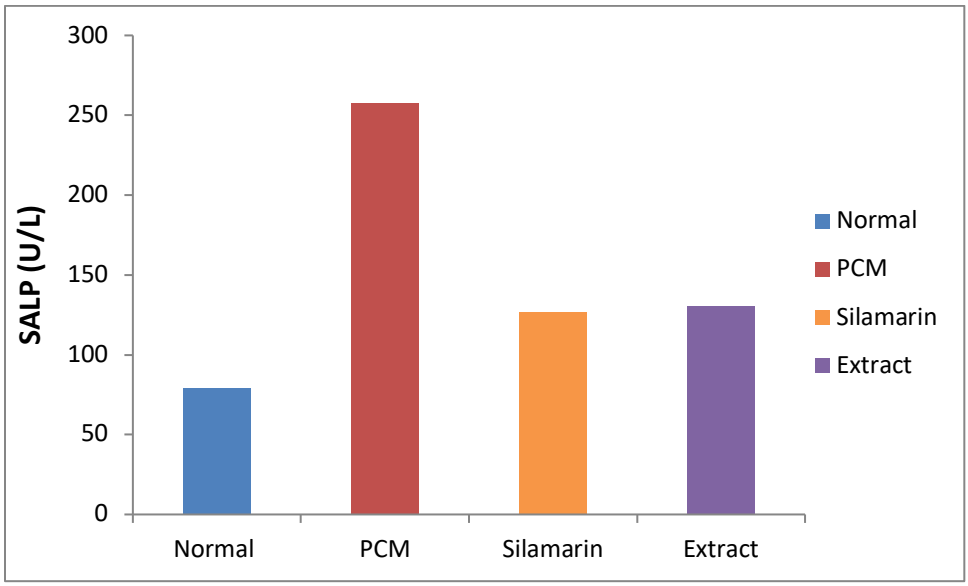


Figure 3: Effect of extracts of Prosopis Cineraria seed extracts on SALP levels of paracetamol intoxicated rats

Values are mean ±SEM, n=6, where, SALP- Serum alkaline phosphatase.

EEPC 400 indicates Ethanolic Extarct of Prosopis Cineraria seed

SOD:

There was significant (P<0.01) reduction in SOD levels in liver when compared to normal. There was an increase in SOD levels in all the treated groups. EEPC 400 produced significant (P<0.01) increase in SOD levels when compared to control which was better (P<0.01) than silymarin. (Table No. 3 and Fig. No. 4)

Table 3: Effect of Prosopis Cineraria seed extracts on SOD levels in paracetamol induced hepatotoxicity models in rats

Paracetamol induced hepatotoxicity	
Treatment	**SOD(U/mg)**
Normal	12.57 ± 0.51
Control (Paracetamol)	5.74 ± 0.09^{**a}
Silymarin+ Paracetamol	6.33 ± 0.07
EEPC 400+ Paracetamol	10.33 ± 0.15^{**b,**c}

Values are the mean ±SEM, n=6, where, EEPC 400, indicates Ethanolic extract of Prosopis Cineraria SOD- Superoxide Dismutase. *Symbols represent statistical significance.** P < 0.01., *P < 0.05. ‘a’ is comparision of control with normal, ‘b’ is comparision of extracts and silymarin. with control, ‘c’ is comparision of extracts with silymarin. ‘d’ is comparision of aqueous extracts with ethanolic extracts.

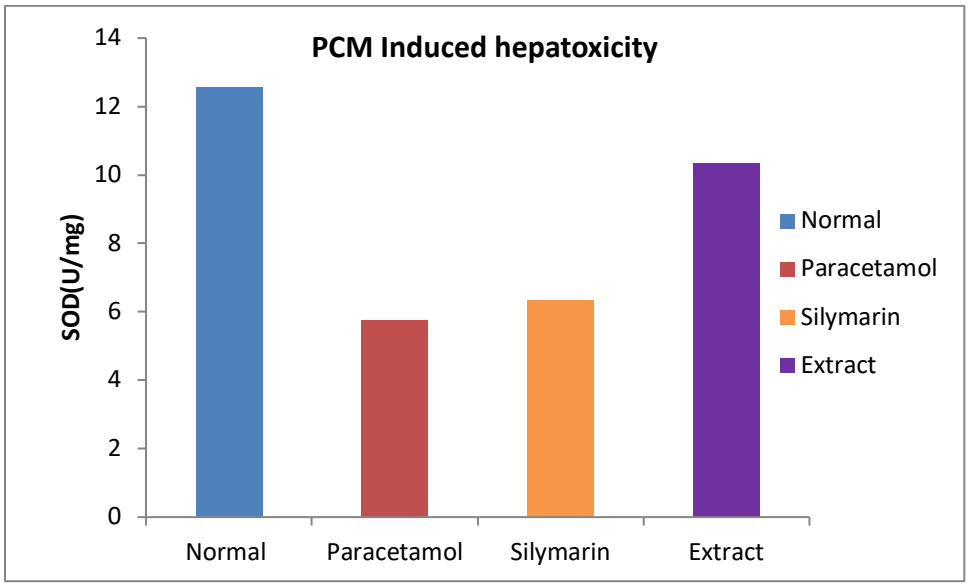


Figure 4: SOD activity following treatment with Prosopis Cineraria seed extracts in liver of paracetamol intoxicated rats

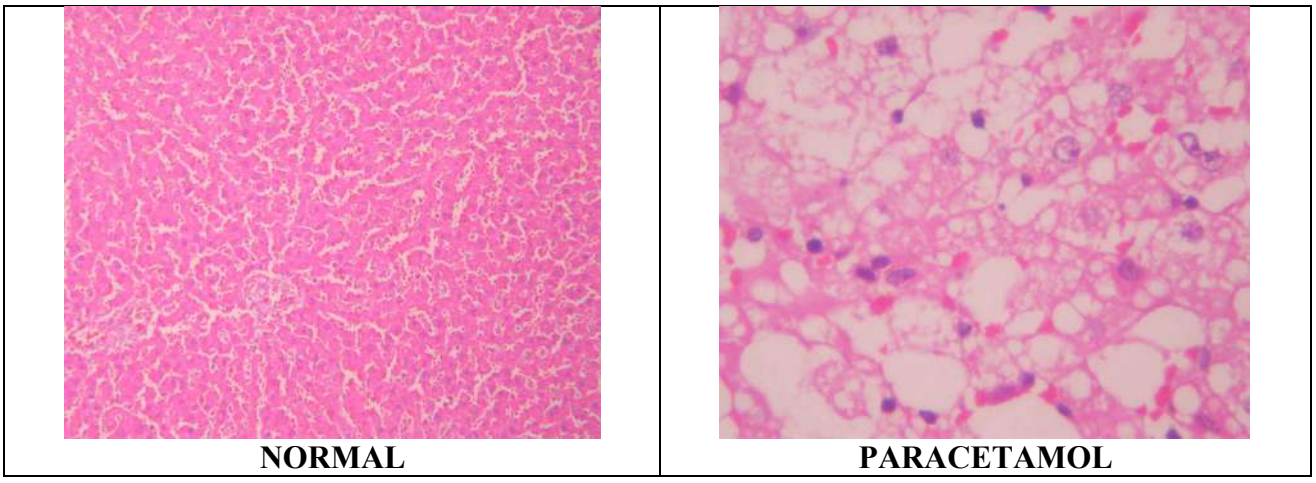
Values are mean ±SEM, n=6, where, SOD is Super oxide dismutase. EEPC 400 indicates Ethanolic Extarct of Prosopis Cineraria seed

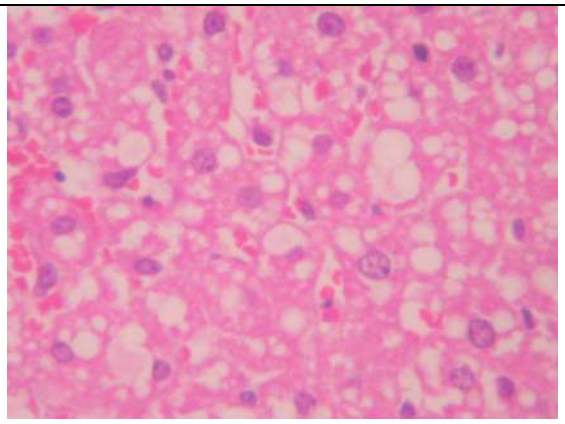
Result of histopathological study:

The histopathological studies conducted support results obtained from the experiments conducted in which the markers of hepatic functions like SGOT, SGPT and SALP were determined (Figure No. 1,2,3).

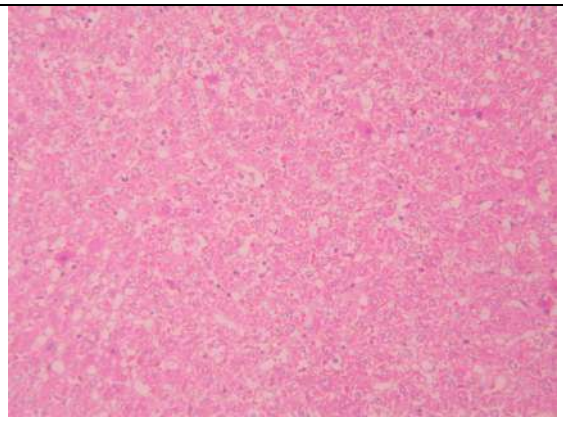
Paracetamol induced hepatotoxicity in rats

Control mice showed normal hepatocytes and hepatic lobules surrounded by central vein, whereas paracetamol treated group was degeneration of hepatocytes, edema, karyohexis, where Ethanolic Extarct of Prosopis Cineraria seed dose showed repaired glomerular and tubular structure.





SILYMARIN



EECP 400

Figure 9: Paracetamol induced hepatotoxicity (Liver sections)

Conclusion: In present investigation that the ethanolic extract of Prosopis Cineraria seeds exhibits significant hepatoprotective activity against paracetamol-induced hepatotoxicity in albino rats. The administration of paracetamol at toxic doses resulted in severe liver damage, as evidenced by marked elevations in serum biochemical markers including SGOT, SGPT, and SALP, along with a significant reduction in hepatic antioxidant enzyme SOD. Histopathological findings further confirmed extensive hepatocellular degeneration, inflammation, and necrosis in the paracetamol-treated group.

Pretreatment with the ethanolic extract of Prosopis Cineraria at a dose of 400 mg/kg body weight significantly reversed the elevated levels of liver enzymes and restored SOD levels, indicating the extract's potent ability to protect liver cells from oxidative damage. The hepatoprotective effect of the extract was found to be comparable, and in some parameters even superior, to the standard hepatoprotective agent silymarin. Histological examination of liver tissues in treated groups showed improved hepatic architecture, reduced necrosis, and preserved cellular integrity, further validating the protective efficacy of the extract.

Phytochemical screening revealed the presence of bioactive constituents such as flavonoids, tannins, saponins, phenolic compounds, and steroids in the ethanolic extract, which are known to possess antioxidant and membrane-stabilizing properties. These constituents likely contribute synergistically to the observed hepatoprotective effects.

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