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HEPATOPROTECTIVE ACTIVITY OF ZIZIPHUS MAURITIANA ROOTS AGAINST CARBON TETRACHLORIDE INDUCED LIVER CIRRHOSIS IN WISTER RATS

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Abstract: Abstract

"Cirrhosis" is a morphologic term that has been used for almost 200 years to denote the end stage of a variety of chronic liver diseases. The term implies a condition with adverse prognosis due to the well-known complications of portal hypertension, hepatocellular carcinoma, and liver failure The study investigates the hepatoprotective effects of an ethanolic extract of Ziziphus mauritiana roots against carbon tetrachloride (CCl4)-induced liver damage in rats. The research involved administrating different doses of the extract (100 mg/kg and 200 mg/kg) to assess its efficacy in mitigating hepatic damage. Results showed significant improvements in liver enzyme levels, total protein, and bilirubin levels, indicating a protective effect. Histological analysis further confirmed the restoration of liver architecture in treated groups. The hepatoprotective properties are attributed to the phytochemical constituents of the extract, particularly flavonoids, which exhibit antioxidant and membrane-stabilizing activities. The findings suggest that Ziziphus mauritiana root extract holds potential as a natural remedy for liver damage, warranting further investigation to elucidate the exact mechanisms and active compounds involved.

Index Terms - Hepatoprotective activity, Ziziphus mauritiana, Silymarin, Carbon tetrachloride (CCl4),Oxidative stress, Liver regeneration.

I. INTRODUCTION

The liver is a vital organ with unique anatomical and functional features, playing a crucial role in infection defense through its extensive reticuloendothelial cell network. It is the second-largest organ and the only one capable of regeneration, comprising 1.5-2.5% of the body's lean weight. The liver, often called the "great chemical factory," is essential for metabolism, secretion, storage, and detoxification. It synthesizes, stores, and secretes essential proteins, nutrients, and chemicals while eliminating toxins. The liver receives about 30% of cardiac output, uniquely sourcing blood from both the portal vein and hepatic artery, crucial for metabolic and energy regulation, including maintaining blood glucose levels during fasting. Bile production and excretion are vital liver functions, facilitated by bile canaliculi formed by hepatocyte membranes. "Cirrhosis" denotes end-stage chronic liver diseases, characterized by liver cell necrosis, fibrosis, and nodule formation, leading to impaired liver function and blood flow, causing complications like portal hypertension and hepatocellular carcinoma. The term, introduced in 1826 by Laennec, derives from the Greek "scirrhus," referring to the liver's orange surface. Cirrhosis involves irreversible liver damage due to excessive extracellular matrix deposition and altered hepatic vasculature, presenting a common endpoint for chronic liver injuries. CCl4 is frequently used to induce liver cirrhosis in animal models. Metabolized by cytochrome P450 into trichloromethyl and trichloromethyl peroxy radicals, CCl4 induces lipid peroxidation, oxidative stress, and subsequent hepatocyte damage, leading to fibrosis and cirrhosis. Silymarin, a hepatoprotective agent, counters these effects by stabilizing membrane permeability and inhibiting lipid peroxidation. It enhances glutathione production, a potent antioxidant, thereby protecting the liver from oxidative stress and promoting tissue repair. Studies show silymarin reduces serum enzyme markers (AST, ALT, ALP) indicative of liver damage, supporting its therapeutic potential against hepatotoxicity.

II. MATERIALS AND METHODS

Experimental animals:
Healthy Wistar albino rats, aged 6-8 weeks and weighing 220±10 g b.w. were obtained from the Vidyabharati College of Pharmacy, Amravati, India (CPCSEA Registration no. 1504/PO/RE/S/11/CPCSEA). The animals were housed in a temperature-controlled (22 ± 3°C) environment with a 12-hour light/dark cycle and had free access to standard rodent chow and water. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) and conducted in accordance with the guidelines of the committee for control and supervision of Experiments on Animals (CCSEA).

Selection of the plant
The medicinal plant Ziziphus mauritiana (Family: Rhamnaceae) was selected for Hepatoprotective activity based on the literature survey.

Preparation of plant extract
The root of Ziziphus mauritiana was processed by washing with clean water, air-drying, pulverizing, and sieving through a 0.3 mm sieve. The powdered material was then placed into a thimble made of stout filter paper and set up in an apparatus. Ethanol was chosen as the solvent and heated in a flask on a water bath. As the solvent boiled, its vapours rose through a side tube into a water condenser, where they condensed and dropped onto the solid material in the thimble. This dissolved the organic substances present in the powdered material, and the solution filtered out into the space between the thimble and the glass cylinder. As the liquid level rose, the solution flowed through a siphon back into the boiling flask. The solvent was vaporized again, leaving the extracted substance behind in the flask. This process facilitated a continuous stream of pure solvent dropping onto the solid material, extracting the soluble substance and returning to the flask. After completion, the solvent in the boiling flask was distilled off, leaving the organic substance behind[].
Subsequently, the ethanolic extract of Z. mauritiana roots was transferred to a clean, dried beaker and concentrated by placing it on a water bath, then cooled and stored in a freezer. From this concentrated extract, preliminary phytochemical screening was conducted following standard scientific protocols to identify the presence of various phytochemical constituents.

Phytochemical screening:
The ethanolic extract of Z. mauritiana roots was screened for the presence of various phytochemicals, including carbohydrates, flavonoids, alkaloids, glycosides, phenolic acids, triterpenoids, sterols, fatty acids, tannins, proteins, and amino acids, using standard qualitative methods.

Drugs and chemicals
Inducing Agent: Carbon Tetrachloride was issued from store of Vidyabharti college of pharmacy,Amravati.
Vehicle for CCL_4: Olive Oil.
Standard drug: Silymarin 70mg tablet manufactured in India by Orion life science and brought from pharmacy store Amravati
Treatment drug: ethanolic extract of Z. mauritiana roots

Methodology
Thirty male albino wistar rats weighing 200- 250 g were selected for the study. Among the 5 groups with 6 animals in each group. Rats were divided into five groups with six animals each. The first group receive oral dose of the vehicle (1ml/kg), the second group receives Carbon Tetrachloride 1ml/kg i.p. Third group received silymarin (100mg/kg), fourth and fifth group receives oral dose of (100 mg/kg and 200 mg/kg respectively.

Instruments and equipment: Centrifuge weighing balance, pipettes, test tube/racks, timer, biochemical analyser, microscope.

Preparation of doses and treatments. The activity of ethanolic extract of Ziziphus mauritiana roots as a hepatoprotective plant using CCl4-induced hepatic damage in mice. Subsequently, CCl4 was administered intraperitoneally at a dose of 1 ml/kg with olive oil as a vehicle. The ethanolic extract of Ziziphus mauritiana roots was administered at doses of 100 and 200 mg/kg, which were selected based on a previous sub-acute toxicity study. Silymarin was administered to animals by gavage.

A) Treatment protocol:

The rats were randomly divided into 5 groups of 6 rats each.

Table 1 : Treatment protocol for all groups:

Sr.no.	Group	No. of Animals	Treatment and Dose	Route of Administration
1	I (Normal control)	6	Saline treatment	IP
2	II (Negative control)	6	Single dose of CCl_4(1 ml/kg/day)	IP
3	III (standard dose)	6	CCl_4 (1 ml/kg/day) + Silymarin (100 ml/kg)	IP and Oral
4	IV (Treatment 1)	6	CCl_4 (1 ml/kg/day) + Moderate dose of Ziziphus mauritiana root extract (100 mg/kg bw)	IP and Oral
5	V (Treatment 2)	6	CCl_4 (1 ml/kg/day) + High dose of Ziziphus mauritiana root extract (200 mg/kg bw)	IP and Oral

Histopathology

Liver tissues from each group were fixed in 10% formalin, processed, and embedded in paraffin. Sections were stained with hematoxylin and eosin (H&E) to assess histopathological changes under a light microscope.

Statistical analysis

The data obtained from the screenings were subjected to statistical analysis following One-way ANOVA followed by Dunnett Comparison Test to assess the statistical significance of the results using GraphPad prism-9 software.The difference was considered significant if $p < 0.05$, moderately significant if $p < 0.01$, and highly significant if $p < 0.001$.

III. RESULTS

- **Phytochemical Screening:**

The phytochemical screening revealed the presence of flavonoids, alkaloids, glycosides, phenolic acids, sterols, tannins, proteins, amino acids, phenols, and saponins in the extract.

Table: Physico-chemical tests of ethanolic extract of Ziziphus mauritiana roots.

Sr. No.	Test	Standards	Results	Method
A	**Physico-chemical tests**			
1	Description	Light colored powder	Complies	Organoleptic
2.	Odour	Characteristics	Characteristics	Organoleptic
3.	Identification	Positive	Complies	TLC
4.	Moisture Content	NMT-9% w/w	1.5%	IP-2020
5.	Ash Content	NMT-6% w/w	3.4%	IP-2020
6.	Acid insoluble Extract	NMT-2% w/w	0.43%	IP-2020
7.	Bulk Density (untapped)	NLT-0.8% g/ml	0.51 g/ml	IP-2020
8.	Bulk Density (tapped)	NMT-0.21g/ml	0.75 g/ml	IP-2020
9.	50% Alcohol Soluble Extractive	NLT-80% w/w	77.2%	IP-2020
10.	Water Soluble Extractive	NLT-70% w/w	33.8%	IP-2020
11.	Particle Size	More than 80% pass through 63 mesh	Complies	63 mesh sieves
12.	pH (1%solution water)	6-7	4.3	IP-2020

❖ Pharmacological evaluation parameters for hepatoprotective:

➢ **Biochemical parameters:**

- **Effect of ethanolic extract of roots of Z. mauritiana on the Body weight, Liver weight and Liver index:**
- Treatment of CCl_4 caused significant reduction ($P<0.0001$) in body weight while increased the absolute liver and relative liver weight comparatively to control group was significantly ($P<0.0001$) restored with 100 mg/kg bw and 200 mg/kg bw treatment of ethanolic extract of roots of Z. mauritiana.
- The normal rats treated with ethanolic extract of roots of Z. mauritiana (200 mg/kg) did not affect the liver weight and liver index compared with those of the normal control rats, indicating that the dose of ethanolic extract of roots of Z. mauritiana may have no liver toxicity in rats. After CCl_4 administration, the liver weight and liver index significantly increased in rats ($p < 0.0001$), indicating serious hepatomegaly that was markedly suppressed by a dose of ethanolic extract of roots of Z. mauritiana (200 mg/kg) and silymarin ($p < 0.0001$).

Table: Effect of ethanolic extract of roots of Z. mauritiana against carbon tetrachloride-induced hepatotoxicity-related parameters in rats (Body weight, Liver weight (g) &Liver index %).

Group	Treatment (n=6)	Body weight (g)	Liver weight (g)	Liver index %
I (Normal control)	Normal control	235.7±0.66	4.58±0.12	1.88±0.06
II (Negative control)	CCl_4	214.6±0.96	7.51±0.16	2.90±0.07
III (Standard dose)	Silymarin	233.2±0.75	5.53±0.10	2.28±0.05
IV (Treatment 1)	Z. mauritiana (100mg/kg)	221.9±0.55	5.76±0.10	2.30±0.04
V (Treatment 2)	Z. mauritiana (200mg/kg)	232.2±0.75	5.63±0.10	2.26±0.07

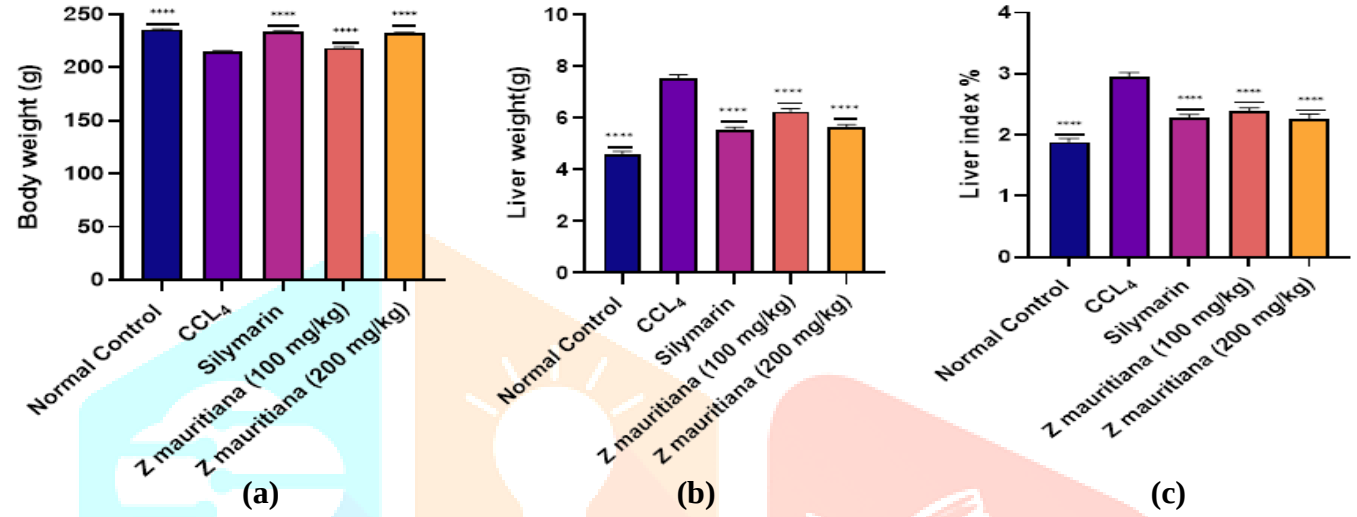


Figure: Effect of CCl_4, Ethanolic extract of Z. mauritiana roots (100 mg/kg, 200 mg/kg), and silymarin (100 mg/kg) on (a)Body weight(g), liver weight (b) and liver index (c). Values are mean ± SEM (n = 6) and analyzed with one-way ANOVA followed by Dunnet's test ***p < 0.0001 vs. CCl_4 group.

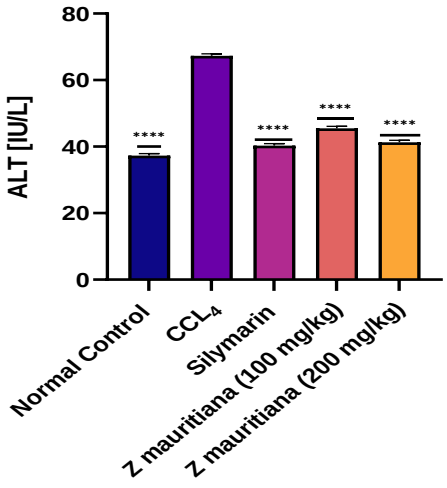
➢ **Effect of ethanolic extract of roots of Z. mauritiana on ALT, AST, ALP & Total protein.**

ALT, AST, and ALP are sensitive markers of the liver, and their elevated levels are indicative of liver damage. As shown in Table , no marked changes of AST, ALT, and ALP levels were detected in normal control rats. The injection of CCl_4 to the rats induced liver injury, which represented markedly elevating activities of AST, ALT, and ALP serum levels compared with the normal control group. However, the ethanolic extract of roots of Z. mauritiana treatment (200 mg/kg) induced a significant ($p < 0.0001$) decrease in the CCl_4-induced elevation of serum enzymes AST, ALT, and ALP compared to the CCl_4-treated group. The effect of ethanolic extract of roots of Z. mauritiana is comparable with that of the silymarin treatment. These results indicated a protective effect of ethanolic extract of roots of Z. mauritiana on CCl_4-induced liver injury in rats.

Total Protein : In CCl_4 intoxicated rats, serum total protein level was decreased significantly ($p < 0.0001$) when compared to the normal control group (Table 11) .The oral administration of ethanolic extract of roots of Z. mauritiana and silymarin reversed the depletion of total protein significantly ($p < 0.0001$) when compared with CCl_4-treated rats.

Table : Effect of CCl_4 toxicity and Ziziphus mauritiana aqueous root extract on enzyme markers and non-enzyme markers of Liver damage.

Group	Treatment (n=6)	ALT(IU/L)	AST (IU/L)	ALP (IU/L)	Total protein (g/dl)
I (Normal control)	Normal control	37.27±0.65	145.2±1.45	127.4±0.87	6.60±0.10
II (Negative control)	CCl_4	67.27±0.65	175.2±1.45	163.7±0.84	3.60±0.10
III (standard dose)	Silymarin	40.27±0.65	164.0±1.41	143.8±1.16	6.25±0.03
IV (Treatment 1)	Z. mauritiana(100mg/kg)	45.50±0.61	170.1±0.49	155.0±1.43	5.06±0.03
V (Treatment 2)	Z. mauritiana(200mg/kg)	41.27±0.65	162.2±0.87	146.8±1.19	6.01±0.04



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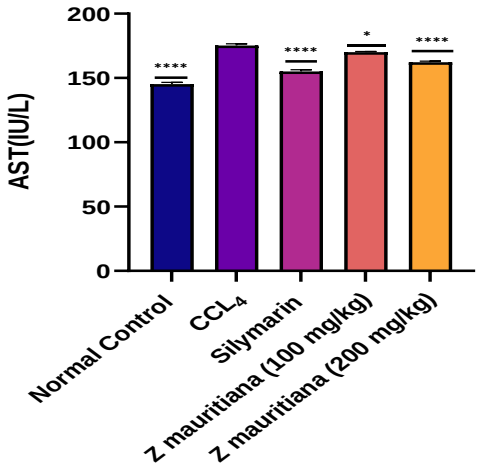

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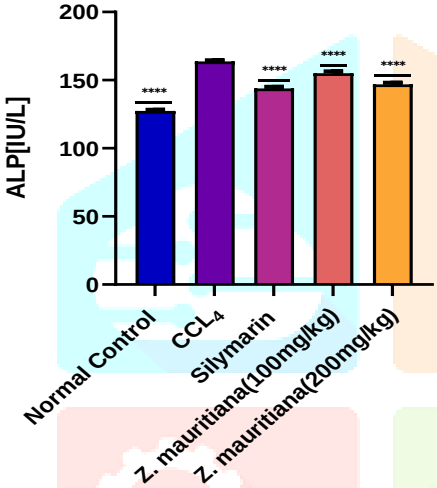

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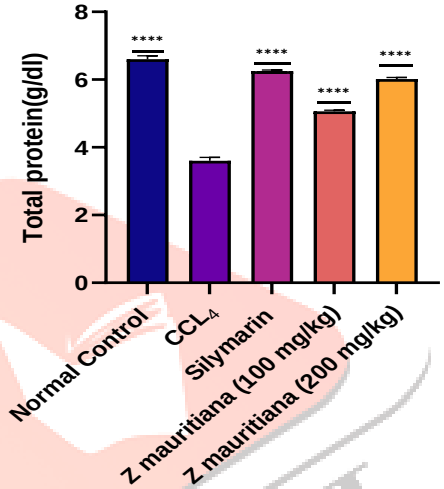


(D)

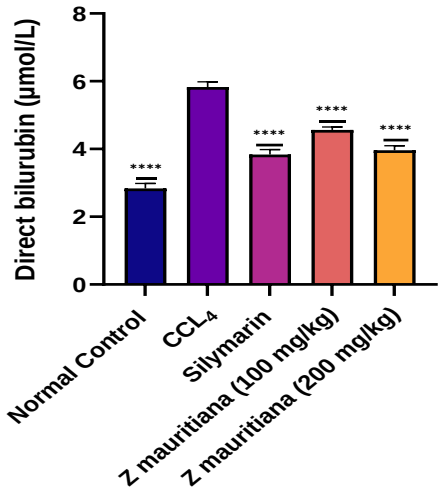
Figure: Effect of CCl_4, Ethanolic extract of Z. mauritiana roots (100 mg/kg, 200 mg/kg), and silymarin (100 mg/kg) on A) ALT B) AST C) ALP D) Total protein. Values are mean ± SEM (n = 6) and analyzed with one-way ANOVA followed by Dunnet's test ****p < 0.0001 vs. CCl_4 group.

- **Effect of ethanolic extract of roots of Z. mauritiana treatment on Total and Direct Bilirubin.**

The administration of CCl_4 to the rats induced a significant ($p < 0.0001$) increase in total and direct bilirubin levels, indicating the impaired excretory function of the liver. On the other hand, treatment with ethanolic extract of roots of Z. mauritiana treatment at a dose of 100mg/kg, 200 mg/kg and silymarin (100 mg/kg) produced a highly significant ($p < 0.0001$) fall in the total and direct bilirubin levels compared to the CCl_4-treated rats.

Table : Effect of ethanolic extract of roots of Z. mauritiana against carbon tetrachloride-induced hepatotoxicity-related parameters in rats (Direct bilirubin &Total bilirubin)

Group	Treatment(n=6)	Direct Bilirubin (µmol/L)	Total Bilirubin (µmol/L)
I (Normal control)	Normal control	2.83±0.15	2.74±0.009
II (Negative control)	CCl_4	5.83±0.15	5.74±0.009
III (standard dose)	Silymarin	3.83±0.15	3.74±0.009
IV (Treatment 1)	Z. mauritiana(100mg/kg)	4.56±0.08	3.91±0.005
V (Treatment 2)	Z. mauritiana(200mg/kg)	3.96±0.13	3.75±0.009



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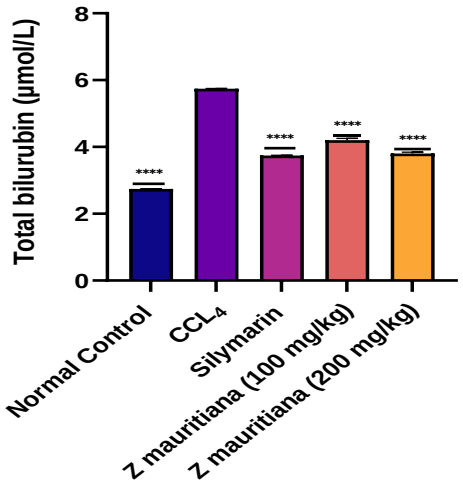

(b)

Figure : Effect of CCl_4, Ethanolic extract of Z. mauritiana roots (100 mg/kg, 200 mg/kg), and silymarin (100 mg/kg) on a)Direct bilirubin b)Total bilirubin . Values are mean ± SEM (n= 6) and analyzed with one-way ANOVA followed by Dunnet's test ∗∗∗p < 0.0001 vs. CCl_4 group

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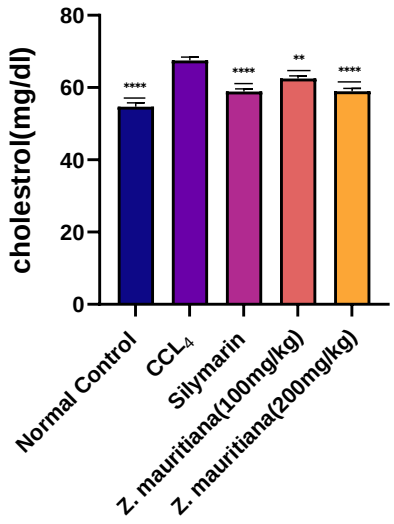
- **Effect of ethanolic extract of roots of Z. mauritiana on Total Cholesterol, Triglycerides, VLDL-c ,LDL, HDL, and Plasma Glucose.**

Administration of CCl_4 alone to the animals resulted in a marked increase in the plasma glucose, triglycerides, VLDL, LDL, Total cholesterol levels ($p < 0.001$) when compared to the normal control group. The rats treated with ethanolic extract of roots of Z. mauritiana treatment at a dose of 100mg/kg, 200 mg/kg and silymarin (100 mg/kg), showed a significant reduction in all of the parameters that were increased in the CCl_4-treated group. Overall, the results observed after administration of ethanolic extract of roots of Z. mauritiana treatment at a dose of 100mg/kg, 200 mg/kg were comparable to those of silymarin at 100 mg/kg.

Table: Effect of ethanolic extract of roots of Z. mauritiana on CCl_4-induced lipid profile changes in rats

Group	Treatment (n = 6)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Glucose (mg/dl)
I (Normal control)	Normal control	54.67±1.11	35.33±0.88	35.73±0.66	66.0±0.96	14.43±1.06	106.8±0.94
II (Negative control)	Inducing	67.50±0.92	65.33±0.88	17.25±0.73	96.0±0.96	24.78±0.92	136.8±0.94
III (standard dose)	Standard	58.87±0.75	45.33±0.88	33.22±0.75	70.0±0.68	16.43±1.06	116.8±0.94
IV (Treatment 1)	T1	62.52±0.74	50.12±0.80	31.22±0.75	79.6±0.61	18.85±1.03	126.2±0.70
V (Treatment 2)	T2	58.95±0.83	47.17±0.83	32.22±0.75	72.0±0.68	17.17±1.04	118.0±0.96



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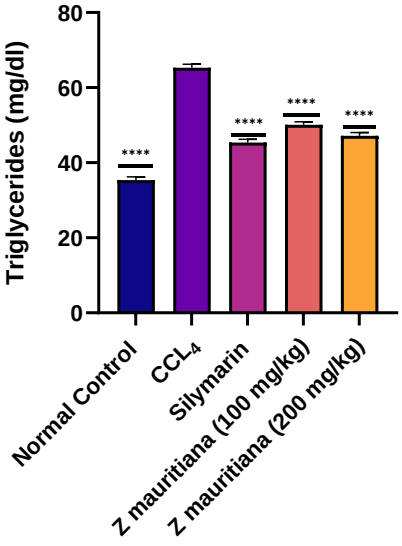

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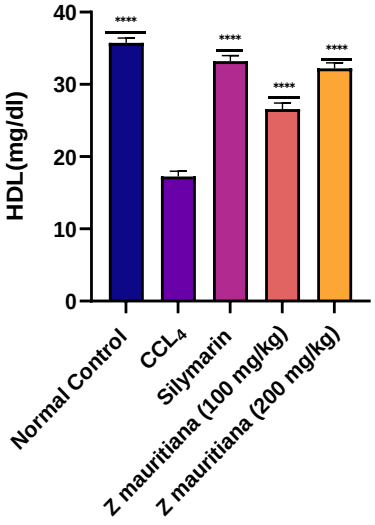

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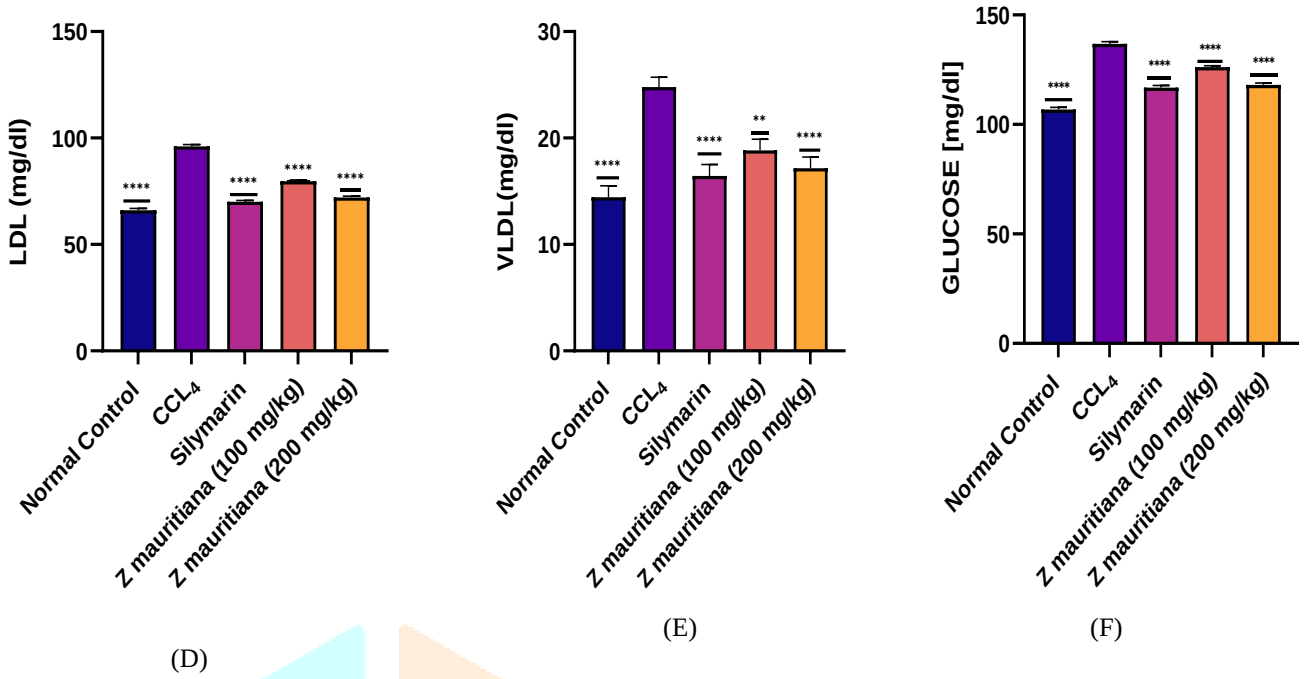

(D) (E) (F)

Figure: Effect of CCl_4, Ethanolic extract of Z. mauritiana roots (100 mg/kg, 200 mg/kg), and silymarin (100 mg/kg) on.(A)cholesterol (B)Triglycerides (C)HDL (D)LDL (E)VLDL(F)Glucose. Values are mean ± SEM (n = 6) and analyzed with one-way ANOVA followed by Dunnet's test ****p < 0.0001 vs. CCl_4 group.

- **Effect of ethanolic extract of roots of Z. mauritiana against carbon tetrachloride-induce kidney function test in serum.**

Table: Effect of ethanolic extract of roots of Z. mauritiana against carbon tetrachloride induce kidney function test in serum.

Group	Treatment(n=6)	Creatinine(mg/dl)	Urea(mg/dl)	Uric acid (mg/dl)
I (Normal control)	Normal control	0.53±0.01	55.33±1.14	1.08±0.02
II (Negative control)	CCl_4	0.83±0.01	75.33±1.14	1.37±0.02
III (standard dose)	Silymarin	0.63±0.01	64.00±0.96	1.10±0.02
IV (Treatment 1)	Z. mauritiana(100mg/kg)	0.70±0.01	68.83±1.16	1.14±0.02
V (Treatment 2)	Z. mauritiana(200mg/kg)	0.65±0.01	65.00±0.95	1.11±0.02

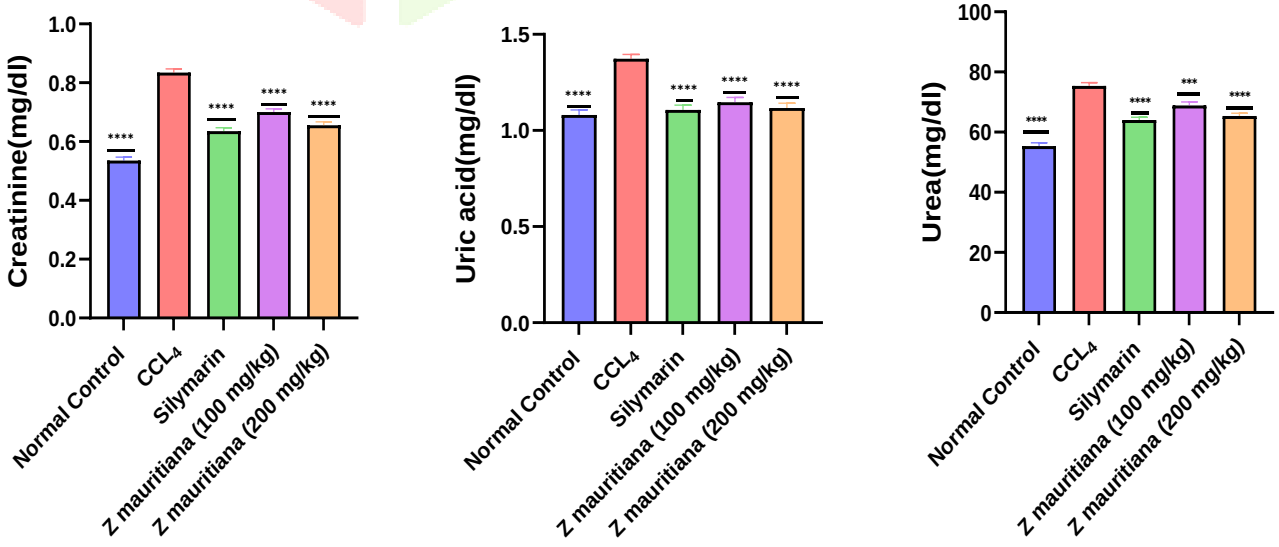

Figure : Effect of CCl_4, Ethanolic extract of Z. mauritiana roots (100 mg/kg, 200 mg/kg), and silymarin (100 mg/kg) on urea , creatinine, uric acid . Values are mean ± SEM (n = 6) and analyzed with one-way ANOVA followed by Dunnet's test ****p < 0.0001 vs. CCl_4 group.

- **Histology :**

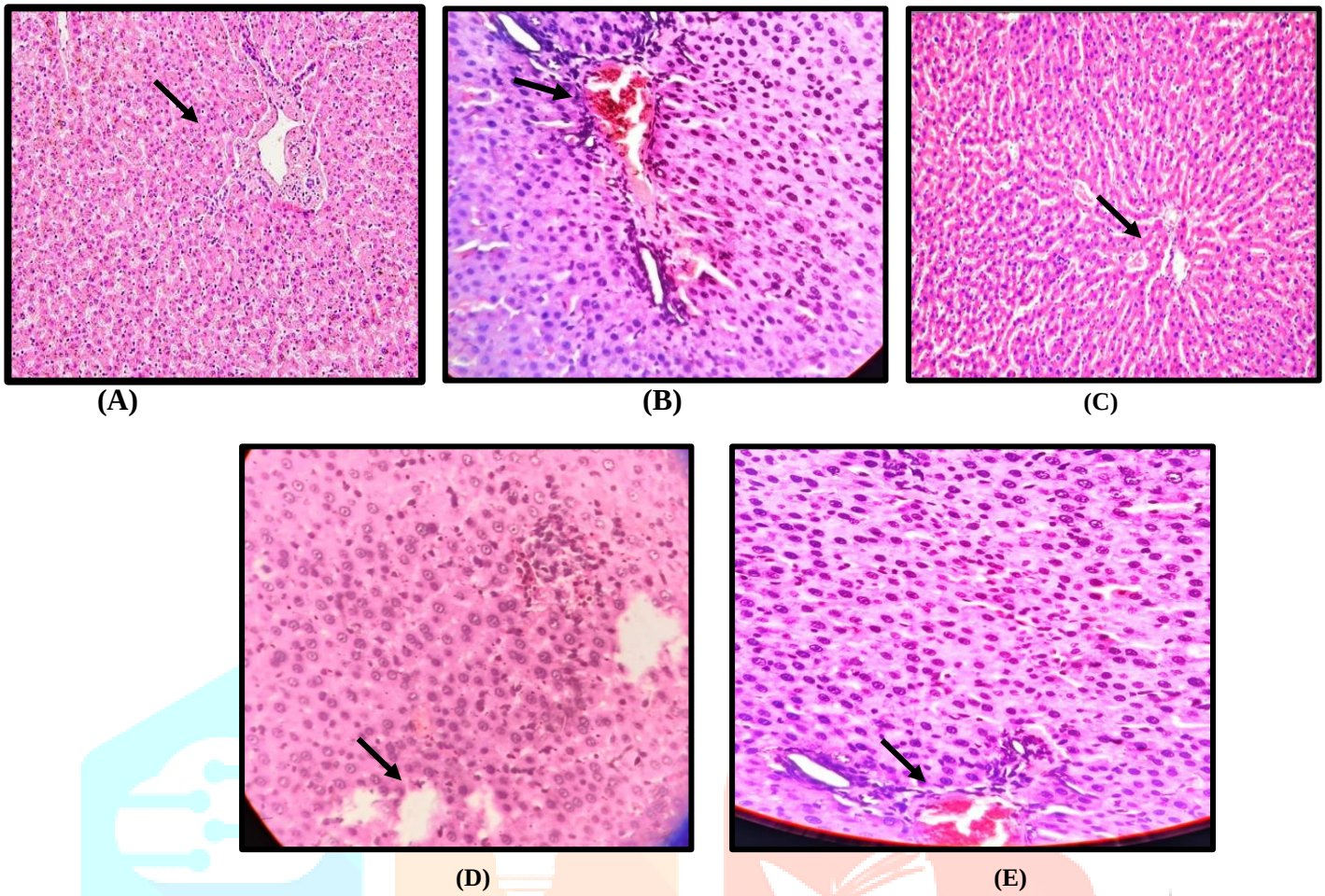

Figure: (A) Control group displaying a normal hepatic architecture without any obvious lesions or histological changes. There were no signs of hemorrhage and the blood vessels were normal.
(B) The group treated with CCl4 exhibited notable congestion of the sinusoids, hepatocyte enlargement, and visible lesions.
(C) The rats treated with CCl_4 + Silymarin (100 mg/kg) demonstrated a notable improvement in the liver's section structure.
(D) CCl_4 + ethanolic Z root extract. Rats given mauritiana (100 mg/kg) showed a slight hepatic structural lesion.
(E) CCl_4 +ethanolic Z root extract. Rats given mauritiana (200 mg/kg) showed a gradual improvement in the liver's structure.

IV. DISCUSSION

Globally, liver disease is the leading cause of morbidity and mortality due to metabolic disorders. Hepatoprotective medicinal herbs have therefore drawn a lot of interest from researchers. Alcohol, the environment, chemicals, and drugs are some of the main causes of liver damage. Hepatotoxic substances cause damage to the liver by elevating oxidative stress, lipid peroxidation, and liver enzymes. Some of the short- to long-term side effects of the sophisticated medications used to treat liver diseases include nausea, vomiting, fatigue, xerostomia, constipation, inflammation, and even death. An important area of research is the examination of medicinal plants to find safer therapeutic or healing effects that come from their natural sources. Throughout history, medicinal plants have been utilized as traditional medicines to treat a variety of illnesses on a global scale. Research has demonstrated the potential therapeutic benefits of certain plants for liver issues.

The hepatoprotective effect of CCl_4 is frequently investigated in animal models, particularly in mice, rats, and rabbits. Lipid peroxidation and oxidative stress are the two main biomarkers for liver damage. Significant changes in the cellular processes that cause inflammation, necrosis, fibrosis, and cirrhosis are part of the pathology. The production of lipid peroxidation, a decrease in the activity of antioxidant enzymes, and an increase in the production of free radicals are the mechanisms by which CCl_4 causes hepatic damage. The enzyme cytochrome P450 is in charge of converting CCl_4 into $CC1_3$ radicals. After that, oxygen and the hazardous metabolite $CC1_3$ radical combine to form the chloromethyl peroxy radical. These radicals cause the lipid membrane of hepatocytes to peroxidatively degrade by binding covalently to macromolecules. As established biomarkers of hepatic damage, our study showed that CCl_4 significantly increased the activities of AST, ALT, and ALP. On the other hand, ethanolic extract of Ziziphus mauritiana + CCl_4-treated rats showed a significant decrease in plasma activities of AST, ALT, and ALP when compared to the CCl_4-treated group. This drop in serum transaminase activity levels is consistent with the generally held belief that transaminases activities return to normal as a result of plasma membrane stabilization and CCl_4-induced hepatic tissue damage repair. This result implies that Ziziphus mauritiana ethanolic extract shielded the liver tissue from damage caused by CCl_4. Additionally, Ziziphus mauritiana ethanolic extract improved the liver's excretory function, as evidenced by a suppression of the rise in serum levels of bilirubin (direct and total). Rats given CCl_4 alone experienced higher plasma glucose levels than the normal control group, owing to the stabilization of the endoplasmic reticulum, which led to protein synthesis. This resulted in a decrease in total protein levels. This elevation could result from the breakdown of glycogen into glucose in hepatocytes following CCl_4 treatment, which raises plasma glucose concentration, or it could be caused by the death of liver cells or disruption of glycogen storage. In contrast to the CCl_4-treated group, rats treated with an ethanolic extract of Ziziphus mauritiana + CCl_4 exhibited a significant decrease in plasma glucose concentration. According to our research, ethanolic extract of Ziziphus mauritiana could improve insulin secretion and

encourage the peripheral glucose uptake . to store glucose. In terms of the lipid profile, rats treated with CCl_4 had significantly higher serum levels of triglycerides and VLDL-c. Prior research has suggested that CCl_4 intoxication-induced disruption of lipid metabolism is the cause of elevated VLDL. Hepatic steatosis is caused by an accumulation of triglycerides in the cytoplasm of hepatocytes. Treatment with an ethanolic Ziziphus mauritiana extract, however, reduced this elevation.. By bringing serum triglycerides (TG) and VLDL levels back to normal levels in comparison to the CCl_4-treated group, this effect suggests that the extract enhanced metabolic function. Hepatomegaly was the other lesion related to the hepatic injury. Liver weight differences resulted from removing individual variation, even though liver index served as an objective measure of hepatomegaly. In the current investigation, the liver index considerably increased in the group receiving CCl_4treatment, suggesting that CCl_4 was the cause of the hepatic damage and hepatomegaly. Nevertheless, the liver weight and liver index were nearly returned to the normal group's levels following treatment with an ethanolic extract of Ziziphus mauritiana (200 mg/kg).

Conversely, the investigation demonstrated that, in contrast to the normal control group, the kidney's biochemical parameters did not exhibit any variation (non-significant). The hepatoprotective effect of ethanolic extract of Ziziphus mauritiana roots against carbon tetrachloride (CCl_4) was demonstrated by these results.

Histological analysis of the liver sections showed that, in contrast to the normal group, hepatotoxin intoxication in the CCl_4 treated group disrupted the normal liver architecture. When ethanolic extract of Ziziphus mauritiana roots and CCl_4 (100 mg/kg and 200 mg/kg) were combined, the exposed animals demonstrated the recovery of damaged cells by maintaining the normal arrangement of the central vein, the radiating pattern of cell plates, and the absence of fat droplets when compared to the hepatocytes of CCl_4damaged groups. Additionally, group C animals (Silymarin + CCl_4) showed further regeneration of hepatic cells. The ethanolic extract of Ziziphus mauritiana roots' effectiveness in lessening liver damage brought on by CCl_4 intoxication is further supported by the histological observations.

Ziziphus mauritiana is known for its unique phytochemical constituents, which include alkaloids, glycosides, triterpenoids, flavonoids, tannins, and saponins. It is known that phytoconstituents with hepatoprotective properties include flavonoids, glycosides, triterpenoids, alkaloids, and saponins. We propose that some of these components and/or other phytochemical compounds may be responsible for the hepatoprotective activity of the ethanolic extract of Ziziphus mauritiana roots, as flavonoids have been known for their antioxidant and antiperoxidant properties leading to hepatoprotective activities do. To pinpoint the precise mechanism(s) underlying the hepatoprotective effects of the ethanolic extract of Ziziphus mauritiana roots, more research is necessary. Additionally, phytochemical analyses are necessary to identify the active ingredients causing this activity.

V. CONCLUSION

The regenerative role of Ziziphus mauritiana root extract induced by CCl_4 toxicity in rats was clarified by the current study, in conclusion. Based on our research, it appears that the ethanolic extract of Ziziphus mauritiana root, particularly when taken in high quantity, exhibits antioxidant activity similar to that of silymarin, the reference antioxidant employed in this investigation. Additionally, the results we obtained indicate that the extract may have positive effects, possibly as a result of the presence of flavonoids that have antiperoxidative and membrane-stabilizing properties. This finding indicates that the flavonoids and tannins in the Ziziphus mauritiana root ethanolic extract may effectively boost the liver's capacity for regeneration and repair. While our study found that the ethanolic extract of Ziziphus mauritiana root has a hepatoprotective effect similar to that of silymarin, more research is necessary to clarify the hepatoprotective mechanism and identify the active ingredients in the Histopathological analysis shows extensive hepatocellular damage, as represented by the presence of portal inflammation, fatty change and venous congestion. Accordingly, our findings may play a role towards the discovery of a new naturopathic remedy.

VI. Future Prospective

Future research on Ziziphus mauritiana root extract should focus on elucidating the specific bioactive compounds responsible for its hepatoprotective effects. Advanced analytical techniques like mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy could be utilized to identify and characterize these phytochemicals. Additionally, studies should explore the molecular mechanisms underlying the antioxidant and anti-inflammatory properties of the extract to provide a comprehensive understanding of its therapeutic potential.

Clinical trials are essential to evaluate the safety and efficacy of Ziziphus mauritiana extract in human subjects, particularly in individuals with liver diseases such as hepatitis and cirrhosis. These trials should investigate optimal dosing regimens, long-term effects, and possible interactions with conventional medications.

Moreover, integrating Ziziphus mauritiana extract into a standardized formulation could enhance its bioavailability and therapeutic efficiency. Nanotechnology-based delivery systems, such as nanoparticles and liposomes, could be explored to improve the extract's stability and target-specific delivery to liver tissues.

Investigating the synergistic effects of Ziziphus mauritiana with other known hepatoprotective agents like silymarin could also open new avenues for combination therapies, offering more robust protection against liver damage.

In summary, while the current study highlights the promising hepatoprotective potential of Ziziphus mauritiana, further research is necessary to fully realize its clinical applications and establish it as a viable natural remedy for liver diseases.

VII. References

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