

Evaluation of the Hepatoprotective Effect of *Ocimum sanctum* (*Ocimum tenuiflorum*) Seed Extract Against Paracetamol-Induced Hepatotoxicity in Wistar Albino Rats

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Abstract

The present study was undertaken to evaluate the hepatoprotective activity of *Ocimum tenuiflorum* seed extract against paracetamol-induced hepatotoxicity in Wistar albino rats. The seed extract was subjected to preliminary phytochemical screening and acute oral toxicity testing according to OECD guidelines. Rats were divided into six groups, including normal control, paracetamol control, Silymarin standard, and *O. tenuiflorum* extract-treated groups at 100, 200, and 400 mg/kg. Hepatoprotective activity was assessed by measuring serum AST, ALT, ALP, total bilirubin, total protein, and albumin, along with hepatic SOD, CAT, GSH, and MDA levels. Histopathological examination of liver tissue was also performed. Paracetamol produced significant hepatic injury and oxidative stress, whereas treatment with the seed extract significantly improved biochemical and antioxidant parameters in a dose-dependent manner. The 400 mg/kg dose showed the maximum protective effect and produced marked improvement in liver histology. The study concludes that *O. tenuiflorum* seed extract possesses significant hepatoprotective activity, possibly through antioxidant and free-radical scavenging mechanisms.

Keywords: *Ocimum sanctum*, *Ocimum tenuiflorum*, Paracetamol, Hepatotoxicity, Oxidative stress, Antioxidant activity.

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Introduction

The liver is the largest gland in the human body and is located mainly in the right upper quadrant of the abdomen, just below the diaphragm. The falciform ligament separates the right and left

anatomical lobes on the anterior surface, while the porta hepatis serves as the hilum through which the portal vein, hepatic artery, and hepatic ducts enter or leave the liver.

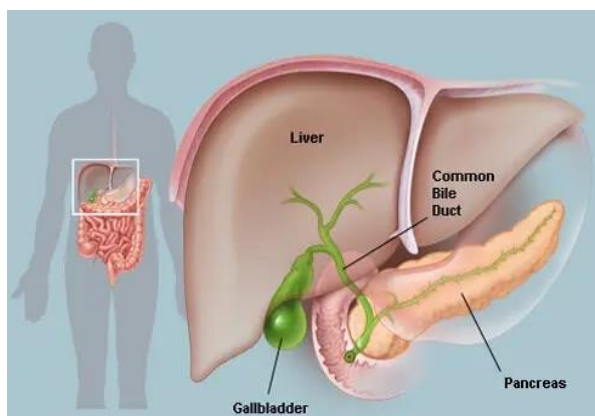


Figure 1: Structure of Liver

The liver has a unique dual blood supply. Approximately 75–80% of hepatic blood flow is supplied by the portal vein, which carries nutrient-rich blood from the gastrointestinal tract, while 20–25% is supplied by the hepatic artery, which provides oxygen-rich arterial blood.

Hepatotoxicity: Hepatotoxicity refers to liver injury caused by exposure to drugs, chemicals, herbal products, or other xenobiotics. The liver is particularly susceptible to toxic injury because it is the primary site for metabolism and detoxification of endogenous and exogenous substances.

Hepatoprotective Study: The increasing incidence of liver diseases and limitations of conventional therapies highlight the need for safer and effective hepatoprotective agents. Medicinal plants rich in

antioxidants and bioactive compounds are widely investigated for their potential to prevent or reverse liver damage.

Plant Profile

Ocimum tenuiflorum: *Ocimum tenuiflorum* L., commonly known as Tulsi or Holy Basil, is an aromatic medicinal plant belonging to the family Lamiaceae. It is widely distributed in India and other tropical regions and holds great importance in traditional systems of medicine such as Ayurveda. The plant is well known for its diverse pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and hepatoprotective properties. Due to its rich phytochemical composition, it has been extensively studied for therapeutic applications.



Figure 2: Plant Profile of *Ocimum tenuiflorum*

Pharmacological Activities: Numerous studies have demonstrated a wide range of pharmacological activities:

- **Antioxidant activity:** Scavenges free radicals and reduces oxidative stress.
- **Antimicrobial activity:** Effective against bacteria, fungi, and viruses.
- **Antidiabetic activity:** Helps in reducing blood glucose levels.
- **Adaptogenic and anti-stress activity:** Enhances resistance to stress.
- **Immunomodulatory activity:** Boosts immune response.
- **Cardioprotective activity:** Improves lipid profile and heart health.

Plant Material: Seeds of *Ocimum tenuiflorum* L. (syn. *Ocimum sanctum* L.) was collected from a Local area and authenticated by a Bilwal Medchem and Research Laboratory Pvt. Ltd. (Ref no:- BMRLIPA/2026/04-07).

Preparation of Extract: A weighed quantity of *Ocimum tenuiflorum* the powder was packed in a Soxhlet apparatus and extracted with methanol for 6–8 hours or until the solvent in the siphon tube becomes colorless. The extract obtained was filtered and concentrated under reduced pressure using a rotary vacuum evaporator.

Acute Toxicity Study: The acute oral toxicity study of *Ocimum tenuiflorum* seed extract was performed as per OECD Guideline 423. The extract was administered orally at the selected starting dose as per OECD 423. At the end of the observation period, the safe dose of the extract was determined, and the doses for hepatoprotective activity was selected accordingly.

Induction of Hepatotoxicity: Experimental hepatotoxicity was induced in wistar albino rats by administration of paracetamol at a hepatotoxic dose. In many rat studies, a single oral dose of 2 gm/kg body weight is used to produce acute liver

injury, although lower or moderately different doses are also reported depending on the protocol.

Experimental Animals: Healthy adult wistar albino rats of either sex weighing 150–200gm was elected for the study. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC), and the study was conducted in accordance with CCSEA guidelines for the care and use of laboratory animals.

Experimental Design: Healthy wistar albino rats was divided into six groups, each consisting of six animals.

Group I: Normal control - Animals will receive normal saline/vehicle only.

Group II: Toxic control - Animals will receive paracetamol 2gm/kg to induce hepatotoxicity.

Group III: Standard group - Animals will receive standard drug Silymarin (50 mg/kg, p.o.) + paracetamol.

Group IV: Test group I - Animals will receive *Ocimum tenuiflorum* seed extract (100 dose) + paracetamol.

Group V: Test group II - Animals will receive *Ocimum tenuiflorum* seed extract (200 dose) + paracetamol.

Group VI: Test group III - Animals will receive *Ocimum tenuiflorum* seed extract (400 dose) + paracetamol.

Biochemical Estimation: The parameters to be estimated will include aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), alkaline phosphatase (ALP), total bilirubin, total protein, and albumin.

Elevation of AST, ALT, ALP, and bilirubin is indicative of hepatocellular damage and impaired liver function and Histopathological Examination.

Statistical Analysis: The data was statistically analyzed by using one-way ANOVA followed by Tukey's multiple comparison test.

The statistical analysis was performed using suitable software such as GraphPad Prism.

Results

Preliminary Phytochemical Screening: The methanolic seed extract of *Ocimum tenuiflorum* was subjected to preliminary phytochemical screening for the identification of various bioactive constituents.

Which may be responsible for the hepatoprotective and antioxidant activity of the extract.

Table 1: Preliminary Phytochemical Screening of *Ocimum tenuiflorum* Seed Extract

S. No.	Phytochemical Constituents	Test Performed	Observation	Result
1	Alkaloids	Mayer's test	Cream precipitate formed	+
2	Alkaloids	Dragendorff's test	Orange-brown precipitate formed	+
3	Flavonoids	Shinoda test	Pink/red colour developed	+
4	Flavonoids	Alkaline reagent test	Yellow colour observed	+
5	Tannins	Ferric chloride test	Blue-green colour developed	+
6	Phenolic compounds	Ferric chloride test	Deep bluish-black colour formed	+
7	Saponins	Froth test	Persistent froth observed	+
8	Glycosides	Keller–Killiani test	Brown ring formed at interface	+
9	Steroids	Salkowski test	Reddish-brown colour observed	+
10	Terpenoids	Liebermann–Burchard test	Green colour developed	+

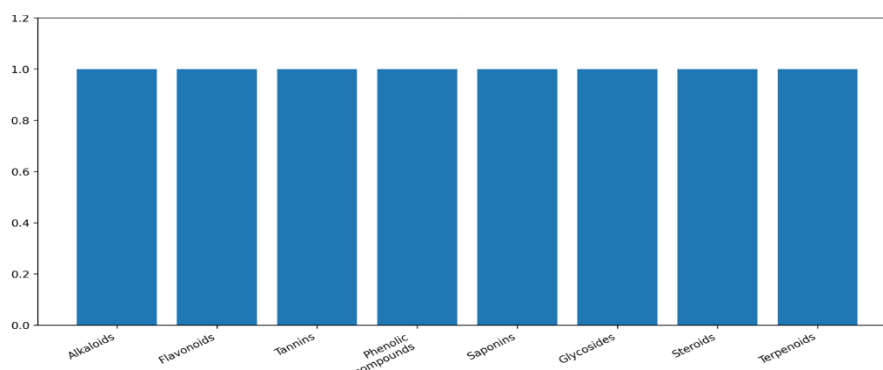


Figure 3: Preliminary Phytochemical Screening of *Ocimum tenuiflorum* Seed Extract

Acute Oral Toxicity Study: The acute oral toxicity study showed that the methanolic seed extract of *Ocimum tenuiflorum* did not produce any mortality

or visible signs of toxicity up to the dose of 2000 mg/kg body weight. This indicates that the extract has a wide margin of safety. Based on the toxicity

findings, lower doses of 100, 200, and 400 mg/kg were selected for the hepatoprotective study.

Effect on Body Weight: Body weight was recorded in all experimental groups at the beginning and at the end of the treatment period.

Treatment with Silymarin and *Ocimum tenuiflorum* seed extract at different dose levels showed improvement in body weight when compared with the toxic control group.

Table 2: Effect of *Ocimum tenuiflorum* Seed Extract on Body Weight in Paracetamol-Induced Hepatotoxicity

Group	Treatment	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)
Group I	Normal Control	162.5 ± 2.4	176.8 ± 2.7	+14.3 ± 1.2
Group II	Paracetamol Control	163.1 ± 2.1	154.2 ± 2.5	-8.9 ± 1.4
Group III	Silymarin + Paracetamol	161.8 ± 2.3	171.5 ± 2.6	+9.7 ± 1.1
Group IV	Extract 100 mg/kg + Paracetamol	162.2 ± 2.0	160.4 ± 2.2	-1.8 ± 1.0
Group V	Extract 200 mg/kg + Paracetamol	161.6 ± 2.5	166.9 ± 2.3	+5.3 ± 1.2
Group VI	Extract 400 mg/kg + Paracetamol	162.0 ± 2.2	170.2 ± 2.4	+8.2 ± 1.0

Values are expressed as Mean ± SEM (n = 6)

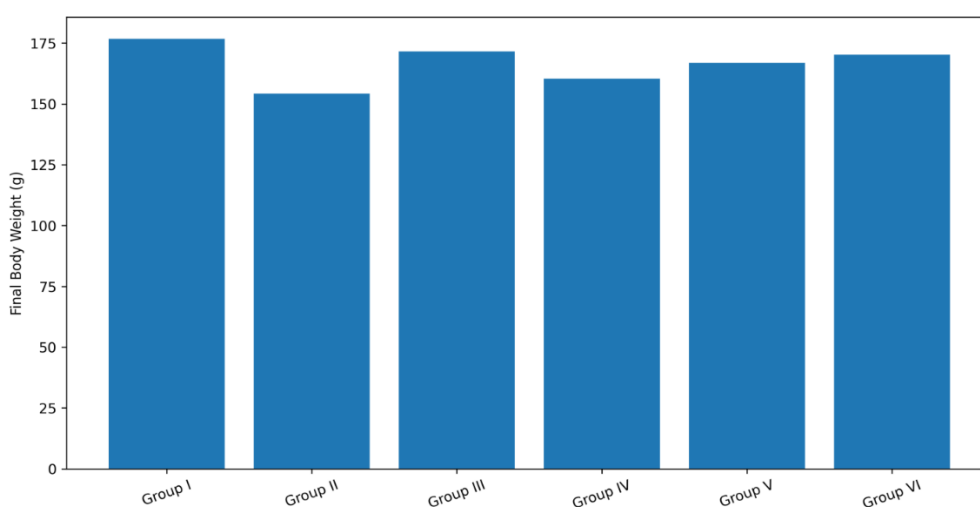


Figure 4: Effect of *Ocimum tenuiflorum* Seed Extract on Body Weight

Effect on Serum Biochemical Parameters: The effect of *Ocimum tenuiflorum* seed extract on serum biochemical parameters in paracetamol-induced hepatotoxic rats. The toxic control group

showed a marked elevation in serum liver marker enzymes such as AST, ALT, ALP, and total bilirubin, along with a reduction in total protein and albumin levels, indicating severe hepatic damage.

Table 3: Effect of *Ocimum tenuiflorum* Seed Extract on Serum Biochemical Parameters in Paracetamol-Induced Hepatotoxic Rats

Group	Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	Total Bilirubin (mg/dL)	Total Protein (g/dL)	Albumin (g/dL)
Group I	Normal Control	78.4 ± 2.5	42.6 ± 1.8	112.3 ± 3.1	0.62 ± 0.04	7.1 ± 0.2	4.2 ± 0.1
Group II	Paracetamol Control	168.7 ± 4.2	124.8 ± 3.6	214.5 ± 5.4	1.86 ± 0.08	4.8 ± 0.2	2.7 ± 0.1
Group III	Silymarin + Paracetamol	91.3 ± 2.9	56.4 ± 2.1	126.8 ± 3.7	0.79 ± 0.05	6.8 ± 0.2	4.0 ± 0.1
Group IV	Extract 100 mg/kg + Paracetamol	140.2 ± 3.8	102.5 ± 2.9	188.4 ± 4.9	1.52 ± 0.07	5.3 ± 0.2	3.0 ± 0.1
Group V	Extract 200 mg/kg + Paracetamol	118.6 ± 3.1	82.7 ± 2.6	156.2 ± 4.1	1.18 ± 0.06	6.0 ± 0.2	3.5 ± 0.1
Group VI	Extract 400 mg/kg + Paracetamol	97.8 ± 2.7	61.3 ± 2.2	132.7 ± 3.5	0.88 ± 0.05	6.6 ± 0.2	3.9 ± 0.1

Values are expressed as Mean ± SEM (n = 6)

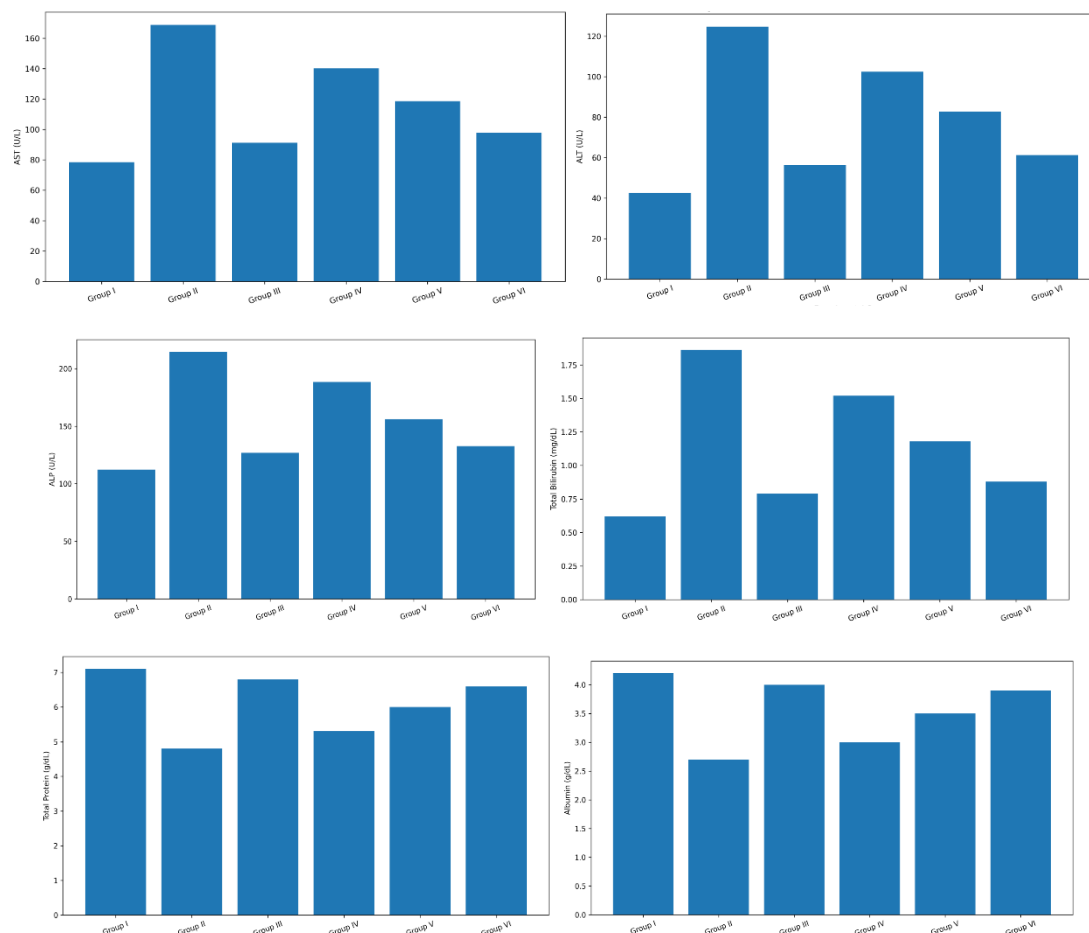


Figure 5: Effect of *Ocimum tenuiflorum* Seed Extract on Different - Different Serum Biochemical Parameters

Treatment with silymarin markedly restored all serum biochemical parameters toward normal values.

Administration of *Ocimum tenuiflorum* seed extract also improved these parameters in a dose-dependent manner. The low-dose group showed mild protection, the medium-dose group showed moderate improvement, and the high-dose group produced marked restoration of enzyme and protein

levels, almost comparable to the standard drug-treated group.

Effect on Antioxidant Parameters: The effect of *Ocimum tenuiflorum* seed extract on antioxidant parameters in liver tissue of paracetamol-induced hepatotoxic rats. Treatment with silymarin and *Ocimum tenuiflorum* seed extract significantly restored these antioxidant parameters toward normal values.

Table 4: Effect of *Ocimum tenuiflorum* Seed Extract on Antioxidant Parameters in Paracetamol-Induced Hepatotoxic Rats

Group	Treatment	SOD	CAT	GSH	MDA
Group I	Normal Control	9.8 ± 0.3	54.2 ± 1.4	8.6 ± 0.2	2.1 ± 0.1
Group II	Paracetamol Control	4.2 ± 0.2	26.8 ± 1.0	3.9 ± 0.2	6.8 ± 0.2
Group III	Silymarin + Paracetamol	8.9 ± 0.3	49.6 ± 1.3	7.9 ± 0.2	2.6 ± 0.1
Group IV	Extract 100 mg/kg + Paracetamol	5.6 ± 0.2	33.4 ± 1.1	5.1 ± 0.2	5.4 ± 0.2
Group V	Extract 200 mg/kg + Paracetamol	7.3 ± 0.3	41.8 ± 1.2	6.5 ± 0.2	4.0 ± 0.1
Group VI	Extract 400 mg/kg + Paracetamol	8.4 ± 0.3	47.2 ± 1.3	7.4 ± 0.2	2.9 ± 0.1

Values are expressed as Mean ± SEM (n = 6)

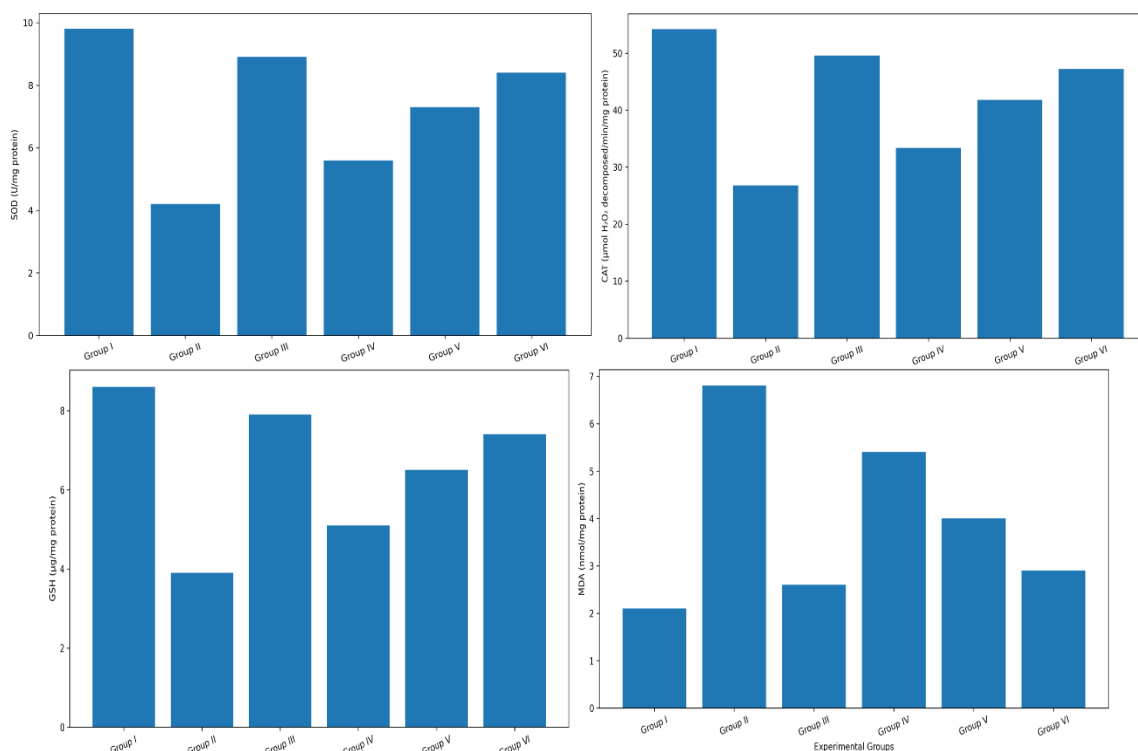


Figure 6: Effect of *Ocimum tenuiflorum* Seed Extract on Antioxidant Parameters

Treatment with silymarin significantly restored antioxidant enzyme levels and reduced MDA level, indicating strong antioxidant and hepatoprotective activity. Administration of *Ocimum tenuiflorum* seed extract also improved antioxidant status in a dose-dependent manner.

The low-dose group showed mild improvement, the medium-dose group showed moderate restoration,

and the high-dose group showed marked recovery of antioxidant parameters, almost comparable to the standard drug-treated group.

Histopathological Study: Histopathological examination of liver sections from different experimental groups showed distinct structural changes corresponding to the degree of hepatic damage and protection.

Table 5: Histopathological Observations of Liver Tissue in Different Experimental Groups

Group	Treatment	Histopathological Observation
Group I	Normal Control	Normal hepatic architecture with intact hepatocytes, normal central vein, and clear sinusoidal spaces
Group II	Paracetamol Control	Severe centrilobular necrosis, hepatocellular degeneration, fatty changes, inflammatory cell infiltration, and sinusoidal congestion
Group III	Silymarin + Paracetamol	Near-normal hepatic architecture with mild degeneration and minimal inflammation
Group IV	Extract 100 mg/kg + Paracetamol	Mild to moderate degeneration with reduced necrosis and inflammatory changes
Group V	Extract 200 mg/kg + Paracetamol	Moderate restoration of hepatic architecture with mild fatty changes and less inflammation
Group VI	Extract 400 mg/kg + Paracetamol	Marked restoration of normal hepatic structure with minimal degeneration and almost normal central vein and hepatocytes

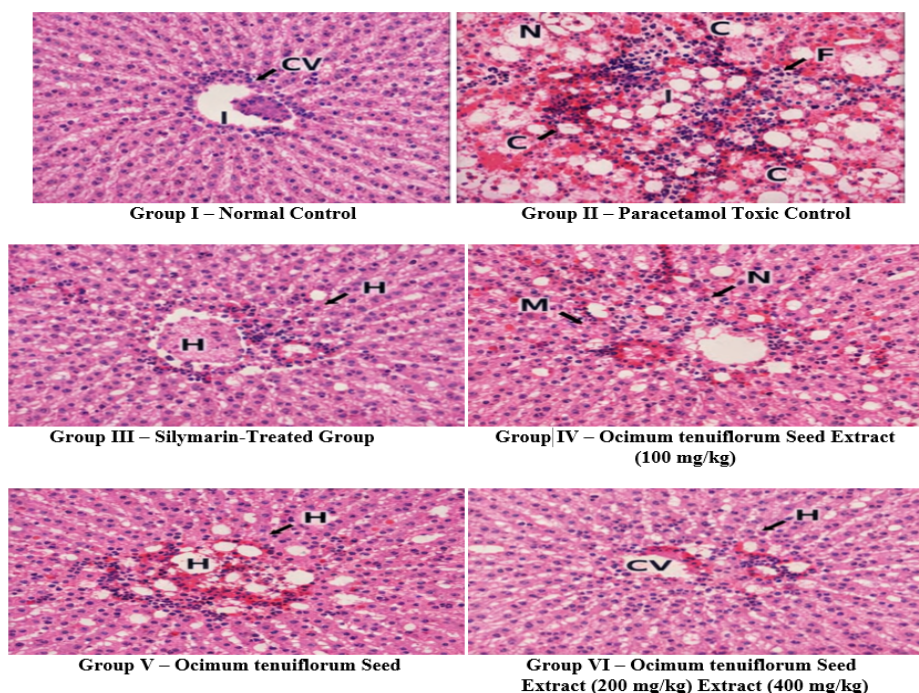


Figure 7: Histopathological Observations of Liver Tissue in Different Experimental Groups

The histopathological findings clearly support the biochemical and antioxidant results. The toxic control group showed severe liver injury due to paracetamol administration, while the standard-treated group showed significant protection. The extract-treated groups showed gradual improvement in liver histology with increasing dose. The high-dose group exhibited the best protective effect among the test groups and showed liver architecture almost comparable to that of the silymarin-treated group.

Discussion

Ocimum tenuiflorum seed extract showed significant hepatoprotective activity against paracetamol-induced liver damage. It improved liver enzyme levels, antioxidant status, and histopathological changes in a dose-dependent manner. The 400mg/kg dose showed the maximum protective effect, comparable to Silymarin.

The activity may be due to the antioxidant properties of its phytoconstituents.

Conclusion

The present study concluded that *Ocimum tenuiflorum* seed extract possesses significant hepatoprotective activity against paracetamol-induced hepatotoxicity in Wistar albino rats. The extract improved biochemical, antioxidant, and histopathological changes in a dose-dependent manner. The 400mg/kg dose showed the maximum protective effect. Therefore, *O. tenuiflorum* seeds may serve as a potential natural source of hepatoprotective agents, mainly through antioxidant and free-radical scavenging activity.

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