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EVALUATION OF THE HEPATOPROTECTIVE AND ANTIOXIDANT ACTIVITIES OF HYDROALCOHOLIC EXTRACT OF IMPATIENS BALSAMINA LINN. LEAVES AGAINST DICLOFENAC-INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT
Drug-induced liver injury is a major health concern and represents one of the most common causes of acute liver failure. Diclofenac, a widely prescribed non-steroidal anti-inflammatory drug (NSAID), is known to induce hepatotoxicity through oxidative stress, mitochondrial dysfunction, and the formation of reactive metabolites. Natural products with antioxidant properties have gained attention as potential hepatoprotective agents. Impatiens balsamina Linn. is a medicinal plant traditionally used for various therapeutic purposes and is rich in flavonoids, phenolic compounds, tannins, and other bioactive constituents with potent antioxidant activity. The present study was undertaken to evaluate the hepatoprotective activity of the hydroalcoholic extract of Impatiens balsamina leaves (HAEIB) against diclofenac-induced hepatotoxicity in experimental rats. HAEIB treatment significantly restored altered biochemical parameters, increased GSH and SOD levels, and reduced LPO levels compared with the diclofenac-treated group, indicating attenuation of oxidative stress. Histopathological examination revealed marked protection against hepatic degeneration, necrosis, and inflammatory cell infiltration. HAEIB at a dose of 400 mg/kg demonstrated hepatoprotective activity comparable to that of the standard drug silymarin. These findings suggest that the hepatoprotective effect of HAEIB may be attributed to its antioxidant phytoconstituents and support its potential use as a natural hepatoprotective agent.

KEYWORDS: Impatiens balsamina, Diclofenac, Hepatotoxicity, Hepatoprotective activity, Oxidative stress, Antioxidant activity, Sprague Dawley rats.

1. INTRODUCTION

The liver is the largest internal organ and plays a pivotal role in metabolism, detoxification, biotransformation of xenobiotics, synthesis of plasma proteins, storage of nutrients, and excretion of endogenous and exogenous substances. Owing to its central role in drug metabolism, the liver is highly susceptible to injury caused by drugs, chemicals, and environmental toxins. Hepatic injury remains a major global health concern and may progress to severe pathological conditions such as hepatitis, fibrosis, cirrhosis, and liver failure. Drug-induced liver injury (DILI) is one of the leading causes of acute liver failure and represents a significant challenge in clinical practice.1,2

Diclofenac, a widely prescribed non-steroidal anti-inflammatory drug (NSAID), is extensively used for the management of pain, inflammation, and musculoskeletal disorders. Although effective, prolonged administration or overdose of diclofenac has been associated with hepatotoxicity. Diclofenac-induced liver injury is primarily mediated through the formation of reactive metabolites, resulting in oxidative stress, mitochondrial dysfunction, lipid peroxidation, inflammatory responses, and subsequent hepatocellular damage. Diclofenac hepatotoxicity has also been associated with immune-mediated mechanisms. These pathological alterations are reflected by elevated serum liver enzymes and impaired hepatic architecture, making diclofenac-induced hepatotoxicity a well-established experimental model for evaluating hepatoprotective agents.3-8

Despite the availability of synthetic drugs for the management of liver disorders, their clinical use is often limited by inadequate efficacy, adverse effects, and high treatment costs. Consequently, there has been increasing interest in medicinal plants as safer and more effective alternatives for the prevention and treatment of liver diseases. Traditional systems of medicine, including Ayurveda, have long utilized herbal remedies for the management of hepatic disorders, and numerous medicinal plants have been scientifically investigated for their hepatoprotective properties.9-11

Impatiens balsamina Linn. (Family: Balsaminaceae), commonly known as Rose Balsam or Jewel Weed, is an annual medicinal herb widely distributed throughout tropical and subtropical regions. The plant has been traditionally used for the treatment of jaundice, inflammation, wounds, rheumatism, burns, skin disorders, and various infectious diseases.8-21 Phytochemical investigations have revealed that Impatiens balsamina contains several biologically active constituents, including flavonoids, phenolic compounds, tannins, alkaloids, terpenoids, coumarins, quinones, saponins, and naphthoquinones.10,11,22-27 These phytoconstituents possess potent antioxidant, anti-inflammatory, antimicrobial, antidiabetic, analgesic, and anticancer activities, suggesting their potential role in protecting tissues against oxidative stress-mediated damage.11,18,20,22-27

Flavonoids and phenolic compounds are well recognized for their ability to scavenge reactive oxygen species, inhibit lipid peroxidation, stabilize cellular membranes, and enhance endogenous antioxidant defense mechanisms. Since oxidative stress is a key contributor to diclofenac-induced hepatotoxicity, the antioxidant-rich phytoconstituents present in Impatiens balsamina may provide significant hepatoprotective effects.19 However, despite the documented pharmacological activities of the plant, scientific evidence regarding the hepatoprotective potential of its hydroalcoholic leaf extract against diclofenac-induced liver injury remains limited.19-24

Therefore, the present study was undertaken to evaluate the hepatoprotective activity of the hydroalcoholic extract of Impatiens balsamina L. leaves against diclofenac-induced hepatotoxicity in experimental rats. In addition, the study investigated the antioxidant potential of the extract by assessing oxidative stress markers and biochemical parameters associated with hepatic injury, thereby providing experimental evidence for its traditional medicinal use.

2. MATERIALS AND METHODS

2.1 Plant Material and Authentication

Fresh leaves of Impatiens balsamina L. were collected from the hilly region of Satara District, Maharashtra, India, during September 2024. The plant material was authenticated by Dr. Vijaya Kumar S, Associate Professor Department of Botany, Sree Siddaganga College of Pharmacy, affiliated with Rajiv Gandhi University of Health Sciences, Karnataka, bangalore India. A voucher specimen (Voucher No. SSCP PAV-10) was prepared and deposited in the Herbarium of the Department of Botany, Government Science College, Satara, for future reference. The collected leaves were washed thoroughly with distilled water to remove adhering dust and foreign matter, shade-dried at room temperature for 10–15 days, and pulverized into coarse powder using a mechanical grinder. The powdered material was passed through sieve No. 40, packed in airtight containers, and stored in a cool, dry place until further extraction.

2.2 Preparation of Hydroalcoholic Extract

The shade-dried and coarsely powdered leaves of Impatiens balsamina (300 g) were extracted using a Soxhlet apparatus with 1000 mL of 70% hydroalcoholic solvent (ethanol water, 70:30 v/v), corresponding to a solvent-to-drug ratio of 3.33:1 (v/w). The extraction was carried out for 12 hours. The obtained extract was filtered and concentrated under reduced pressure using a rotary vacuum evaporator to remove the solvent. The concentrated extract was further dried to obtain a dry brown powder and stored in an airtight container at 4°C until further use. The percentage yield of the extract was calculated using the following equation:

Percentage yield (%) = (Weight of dried extract / Weight of powdered plant material) × 100

2.3 Phytochemical Screening:

a) Qualitative phytochemical analysis:

The hydroalcoholic extract of Impatiens balsamina leaves (HAEIB) was subjected to preliminary qualitative phytochemical screening for the presence of alkaloids, carbohydrates, glycosides, proteins and amino acids, phenolic compounds, tannins, and flavonoids using standard procedures described by Khandelwal and Kokate. The presence or absence of each phytochemical constituent was determined based on the characteristic colour changes or precipitate formation.

b) Quantitative phytochemical analysis:

The total flavonoid content (TFC), total phenolic content (TPC), and total tannin content (TTC) of HAEIB were estimated using standard spectrophotometric methods. TFC was determined by the aluminium chloride colorimetric method using quercetin as the reference standard and expressed as mg quercetin equivalents (QE)/g of dry extract. TPC was estimated by the Folin–Ciocalteu method using gallic acid as the standard and expressed as mg gallic acid equivalents (GAE)/g of dry extract. TTC was determined by the Folin–Denis method using tannic acid as the reference standard and expressed as mg tannic acid equivalents (TAE)/g of dry extract. All analyses were performed using a UV–Visible spectrophotometer. The phytochemical contents were calculated using the following equation:

$$C = xV/m$$

where C is the phytochemical content (mg equivalent/g dry extract), x is the concentration obtained from the respective standard calibration curve (quercetin for TFC, gallic acid for TPC, and tannic acid for TTC) (mg/mL), V is the volume of extract (mL), and m is the mass of the dry extract (g).

2.4 Acute Oral Toxicity Study

The acute oral toxicity study of the hydroalcoholic extract of Impatiens balsamina (HAEIB) was carried out in female Sprague Dawley rats according to OECD Guideline 423 (Acute Oral Toxicity–Acute Toxic Class Method). Animals were

observed for mortality, behavioural changes, and signs of toxicity following oral administration of the extract. Based on the outcome of the acute toxicity study, suitable doses were selected for the hepatoprotective study.

2.5 Ethical Approval and Experimental Animals

The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Rashtrasant Janardan Swami College of Pharmacy, Kokamthan, Kopargaon, Maharashtra, India. The study was conducted in accordance with the guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA), Government of India. Ethical approval was granted under IAEC Approval No. IAEC-I/RJSCOP/2025-2026/05 on 20 March 2026. The institutional animal house is registered with CCSEA under Registration No. 2387/PO/ReRcBi/2026/CCSEA.

Healthy adult Sprague Dawley rats of either sex, 8–10 weeks of age, weighing 180–200 g, were used for the study. The animals were procured from LACSMI Biofarmus Pvt. Ltd., Alephata, Pune, Maharashtra, India. Animals were housed in sanitized polypropylene cages containing sterile paddy husk bedding under standard laboratory conditions (temperature: 23 ± 2°C, relative humidity: 50 ± 5%, and a 12 h light/12 h dark cycle). They were provided with a standard pellet diet and water ad libitum. The animals were acclimatized to the laboratory conditions for 7 days before the initiation of the experiment and were randomly allocated into experimental groups.

2.6 Experimental Design

Healthy adult Sprague Dawley rats of either sex, 8–10 weeks of age, weighing 180–200 g were randomly allocated into five groups, each comprising six animals (n = 6).

- **Group I** (Normal Control): Received normal saline (10 mL/kg, p.o.) for 7 days.
- **Group II** (Toxic Control): Received diclofenac (20 mg/kg, p.o.) for 7 days.
- **Group III** (HAEIB 200): Received HAEIB (200 mg/kg, p.o.) followed by diclofenac (20 mg/kg, p.o.) for 7 days.
- **Group IV** (HAEIB 400): Received HAEIB (400 mg/kg, p.o.) followed by diclofenac (20 mg/kg, p.o.) for 7 days.
- **Group V** (Standard): Received silymarin (50 mg/kg, p.o.) followed by diclofenac (20 mg/kg, p.o.) for 7 days.

All treatments were administered orally once daily for seven consecutive days. Twenty-four hours after the last treatment (Day 8), blood samples were collected from the retro-orbital plexus under light anaesthesia. Serum was separated by centrifugation and used for the estimation of serum biochemical markers. The animals were then sacrificed under an overdose of anaesthesia, and the liver was excised, weighed, and processed for antioxidant assays and histopathological examination.

2.7 Biochemical Estimation

Twenty-four hours after the last treatment, blood samples were collected from the retro-orbital plexus under light anaesthesia. Serum was separated by centrifugation at 3000 rpm for 15 min and analyzed for serum glutamate oxaloacetate transaminase (SGOT/AST), serum glutamate pyruvate transaminase (SGPT/ALT), alkaline phosphatase (ALP), total bilirubin (TB), and total protein (TP) using commercially available diagnostic kits according to the manufacturer's instructions.

2.8 Determination of Wet Liver Weight

Following blood collection, the animals were sacrificed under an overdose of anaesthesia. The liver was excised, washed with normal saline, blotted dry using filter paper, and weighed immediately using an electronic balance.

2.9 Preparation of Liver Tissue Homogenate

Liver tissue (1 g) was homogenized in 10 mL of ice-cold phosphate-buffered saline (PBS) using a Teflon-glass homogenizer to prepare a 10% (w/v) homogenate. The homogenate was centrifuged at 10,000 rpm for 15 min, and the supernatant was used for the estimation of antioxidant parameters.

2.10 Estimation of Antioxidant Parameters

Reduced glutathione (GSH), lipid peroxidation (LPO), and superoxide dismutase (SOD) levels were estimated in the liver tissue homogenate using the methods described by Ellman, Ohkawa et al., and Marklund and Marklund, respectively. The results were expressed as µmol/g tissue for GSH and LPO and U/mg protein for SOD.

2.11 Histopathological Examination

Liver tissues were fixed in 10% neutral buffered formalin, processed by routine paraffin embedding, sectioned at 5 µm thickness, stained with hematoxylin and eosin (H&E), and examined microscopically for histopathological alterations including hepatocellular necrosis, fatty degeneration, inflammatory cell infiltration, and sinusoidal congestion.

2.12 Statistical Analysis

All data were expressed as Mean ± SEM (n = 6). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test using GraphPad Prism software. Differences were considered statistically significant at $P < 0.05$.

3. RESULTS

3.1 Extraction

Table no 1- Percentage yield of Hydroalcoholic extract of Impatiens balsamina (HAEIB)

Solvent	Method of extraction	Physical nature of extract	Color of extract	Percentage yield
Hydroalcoholic 70% v/v (70:30) (Ethanol: distilled water)	Soxhlet extraction	Dry Powder	Brown	13.3%

3.2 Qualitative Phytochemical Screening

Table no. 2 - Qualitative Phytochemical screening Of HAEIB

Sl. No	Chemical constituents	HAEIB
1	Alkaloids	-
2	Glycosides	+
3	Flavonoids	+
4	Phenolics and tannins	+
5	Proteins and amino acids	+
6	Carbohydrates	+
Note: + indicates presence, - indicates absence.		

Qualitative phytochemical screening of HAEIB revealed the presence of glycosides, flavonoids, phenolic compounds, tannins, proteins and amino acids, and carbohydrates, whereas alkaloids were absent.

3.3 Quantitative Phytochemical Analysis:

3.3.1 Estimation of Flavonoid Content

Table no 3 - Estimation of Total Flavonoid Content in HAEIB

Estimation of Total Flavonoid Content in HAEIB		
Quercetin		HAEIB
Conc. (µg/ml)	Absorbance	Absorbance
100	0.0316	0.0037
200	0.0951	0.022
300	0.1717	0.0259
400	0.2125	0.0448
500	0.2453	0.0633
600	0.3052	0.0654
700	0.3237	0.0676

800	0.3828	0.0723
900	0.3993	0.0809
1000	0.4110	0.1020

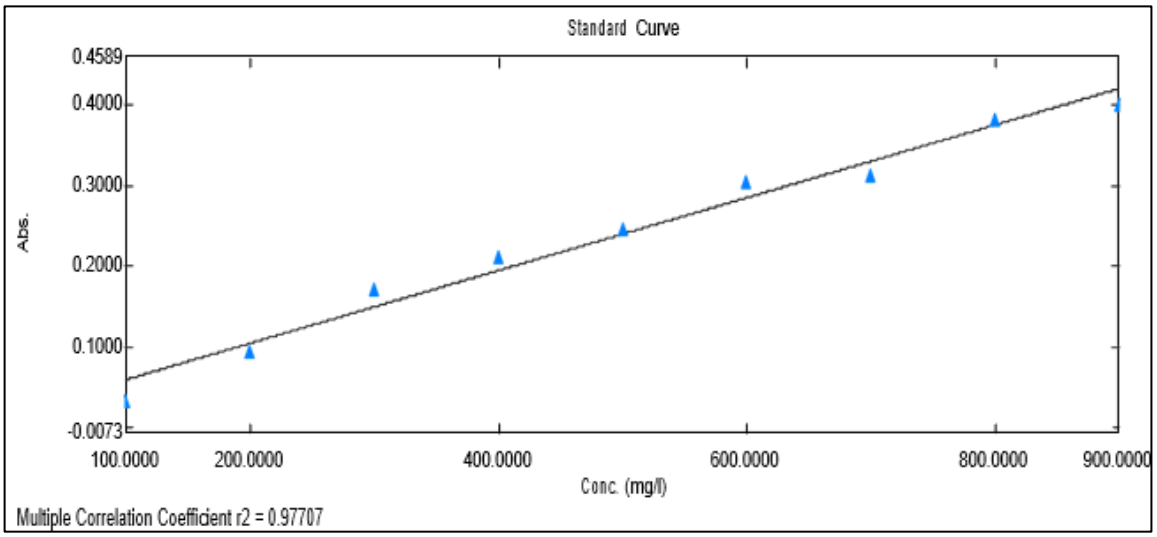


Fig no. 1. Linearity curve of Standard Quercetin

The total flavonoid content of HAEIB was estimated using the aluminium chloride colorimetric method. A calibration curve of quercetin (100–900 µg/mL) was prepared as the reference standard. The total flavonoid content of HAEIB was found to be 180 mg QE/100 g of dry extract.

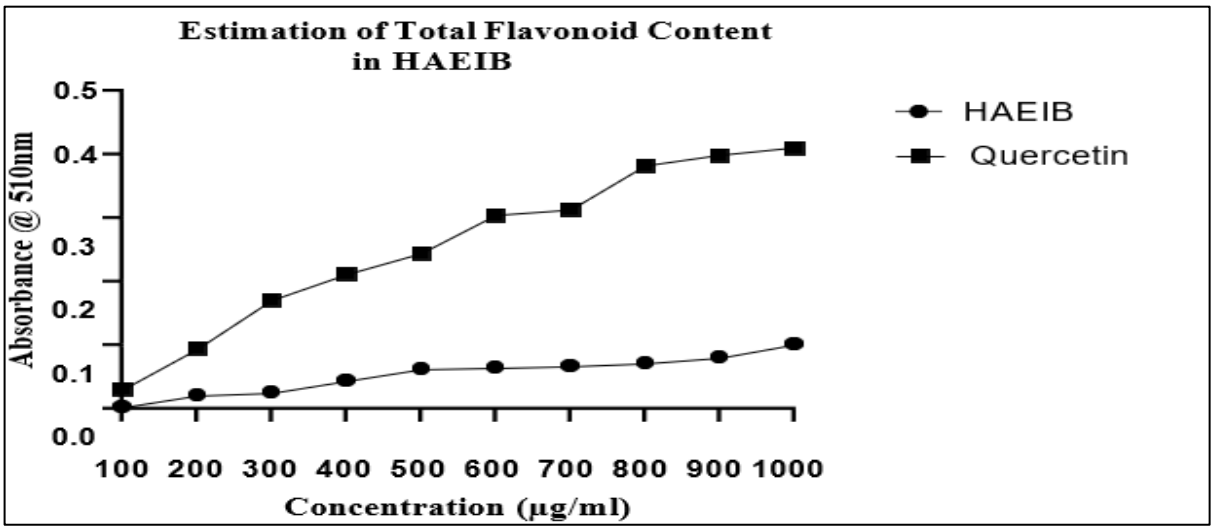


Fig no. 2. Total Flavonoid Content at different concentrations of HAEIB

3.3.2 Estimation of Total Phenolic Content

Table no 4 - Estimation of Total Phenolic content of HAEIB

Gallic acid		**HAEIB**	
Conc. (µg/ml)	**Absorbance**	**Conc. (µg/ml)**	**Absorbance**
10	0.3458	100	0.1471
20	0.4530	200	0.1709
40	0.6004	300	0.1755

60	0.7701	400	0.1983
80	0.9891	500	0.204
100	1.1242	600	0.2296
		700	0.2421
		800	0.246
		900	0.2761
		1000	0.2848

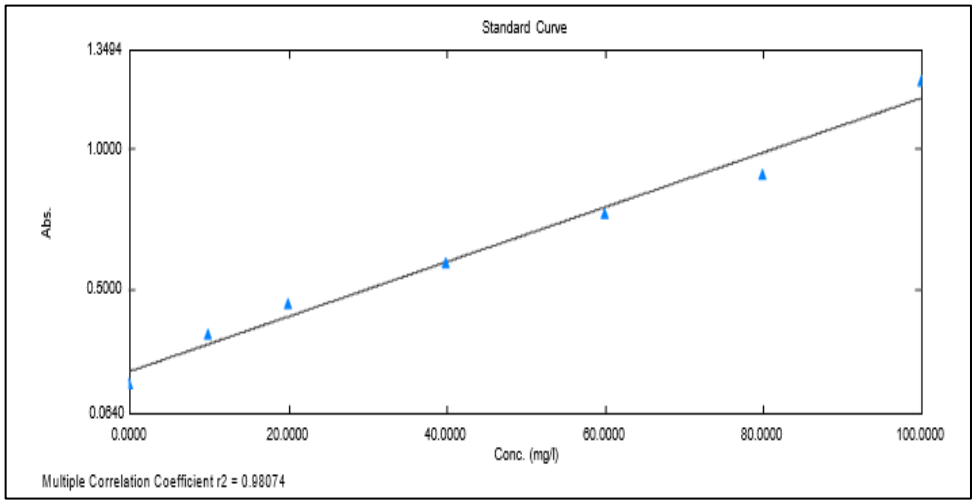


Fig no. 3. Linearity curve of standard Gallic acid

The total phenolic content of HAEIB was estimated using the Folin–Ciocalteu method with gallic acid as the reference standard. The calibration curve of gallic acid (10–100 µg/mL) showed good linearity (R^2 = 0.98074). The total phenolic content of HAEIB was found to be 85.1 mg GAE/100 g of dry extract.

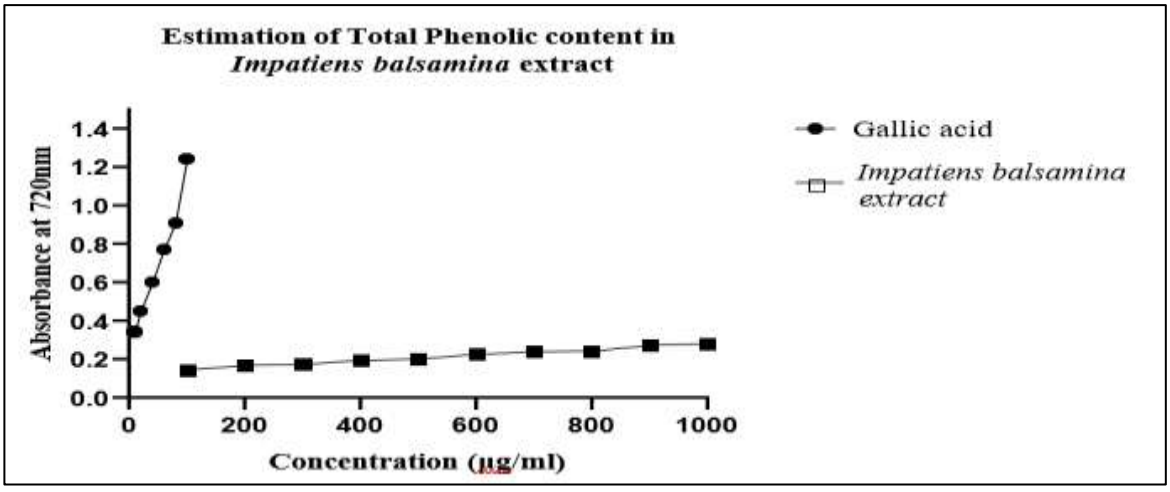


Fig no. 4. Total Phenolic content at different concentrations of HAEIB

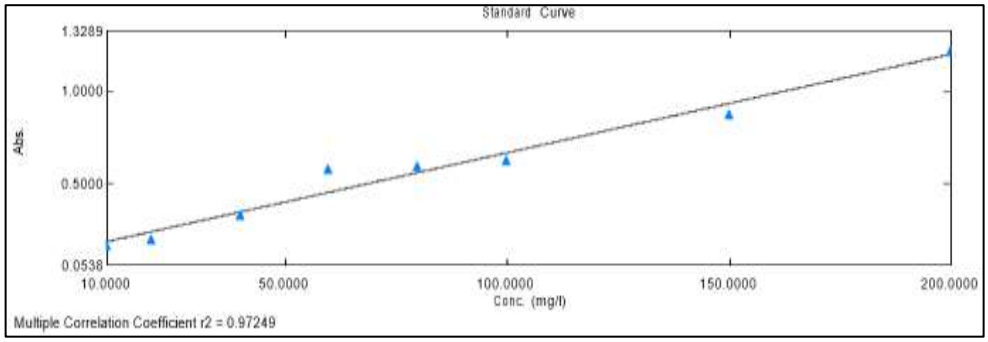


Fig no. 5. Calibration curve of tannic acid for total tannin content estimation.

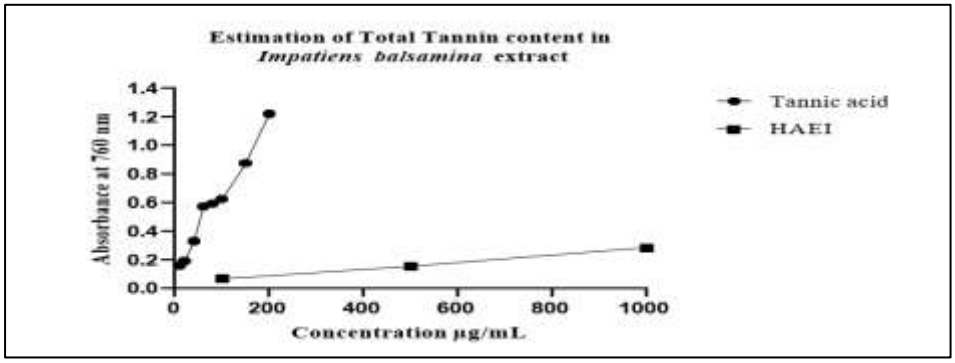


Fig no. 6. Tannin Content at different concentrations of HAEIB

The total tannin content of the hydroalcoholic extract of Impatiens balsamina leaves (HAEIB) was estimated using tannic acid as the reference standard. The calibration curve of tannic acid (10–200 µg/mL) showed good linearity ($R^2 = 0.97249$). The total tannin content of HAEIB was found to be 2.147 mg tannic acid equivalents (TAE)/g of dry extract.

3.4 Pharmacological Investigations

3.4.1 Acute Oral Toxicity Study

The hydroalcoholic extract of Impatiens balsamina leaves (HAEIB) was evaluated for acute oral toxicity according to OECD Guideline 423. No mortality or treatment-related signs of toxicity were observed in either sex rats at an oral dose of 2000 mg/kg during the 14-day observation period. No abnormal changes in behavioural, motor, or neurological functions, or in the appearance of the skin, eyes, and fur were observed. These findings indicate that HAEIB is safe up to an oral dose of 2000 mg/kg under the experimental conditions.

3.4.2 In-vivo studies

i) Effect of HAEIB on Wet Liver Weight

Table no 5 - Effect of HAEIB on Wet liver weight in Diclofenac induced

Groups	Treatment/ dose	Wet liver weight in grams
G1	Normal control	6.08 ± 0.18
G2	Diclofenac alone (20 mg/kg) b.w.p.o.	8.30 ± 0.13####
G3	Diclofenac (20 mg/kg) b.w.p.o.+ HAEIB (200 mg//kg) p.o.	7.20 ± 0.15**
G4	Diclofenac (20 mg/kg) b.w.p.o.+ HAEIB (400 mg/kg) p.o.	6.86 ± 0.21****
G5	Diclofenac 20 mg/kg) b.w.p.o.+ Silymarin (50 mg/kg) p.o.	6.32 ± 0.21****

Diclofenac (20 mg/kg, p.o.) significantly increased the wet liver weight in the disease control group (8.30 ± 0.13 g) compared with the normal control group (6.08 ± 0.18 g). Treatment with HAEIB at doses of 200 mg/kg and 400 mg/kg significantly reduced the wet liver weight to 7.20 ± 0.15 g and 6.86 ± 0.21 g, respectively. Silymarin (50 mg/kg) also

significantly decreased the wet liver weight to 6.32 ± 0.21 g compared with the diclofenac-treated group (Table 5; Figure 7).

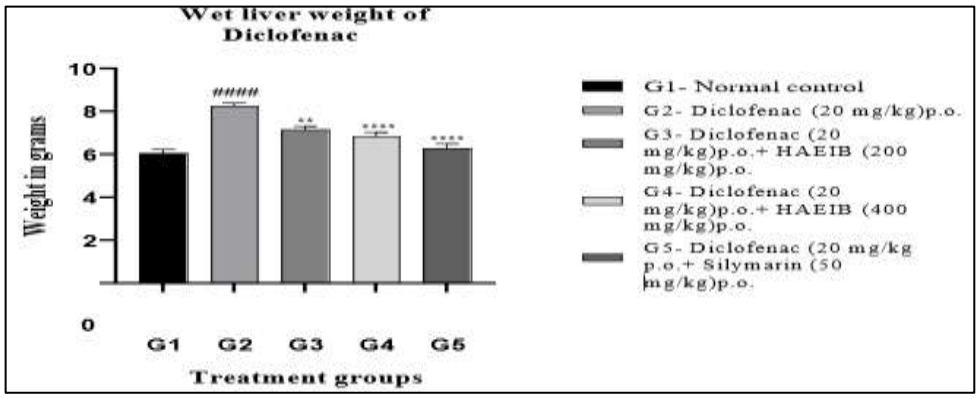
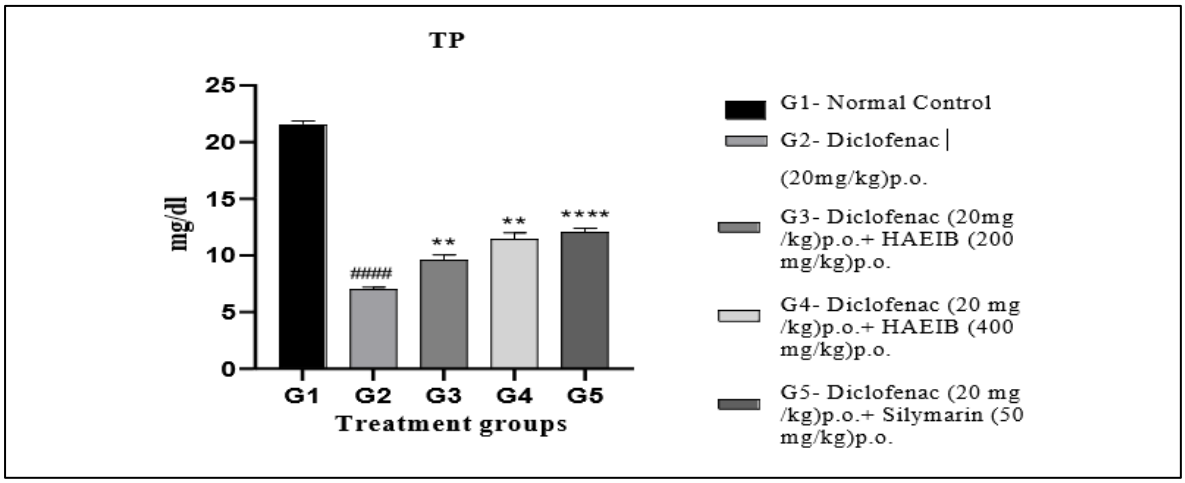


Fig no. 7: Graphical representation of HAEIB on wet liver weight on Diclofenac induced hepatotoxicity in rats.

ii) Serum Biochemical Parameters

Table no 6 - Effect of HAEIB on SGOT, SGPT, ALP, Total Protein and Total Bilirubin levels on Diclofenac induced Hepatotoxicity in rats.

Groups	Treatment	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Total bilirubin (mg/dl)	Total Protein (mg/dl)
G1	Normal	94.49 ± 0.92	0.8800 ± 0.22	128.5 ± 4.86	0.263 ± 0.01	21.60± 0.28
G2	Diclofenac alone(20 mg/kg) p.o.	188.8± 4.03####	2.738±0.05####	382.9±1.30####	1.397± 0.15####	7.092± 0.20####
G3	Diclofenac (20 mg/kg) p.o. + HAEIB (200 mg//kg) p.o.	163.1±3.50****	2.368 ± 0.43	253.2±14.5****	0.670±0.04****	9.663± 0.47**
G4	Diclofenac (20 mg/kg) p.o. + HAEIB (400 mg/kg) p.o.	126.9±2.50****	1.304±0.36**	216.6±30.4****	0.501±0.04****	11.44± 0.63**
G5	Diclofenac (20 mg/kg) p.o. + Silymarin (50 mg/kg) p.o.	115± 3.05****	1.102±0.04**	167.0±6.60****	0.453±0.04****	12.11± 0.35****



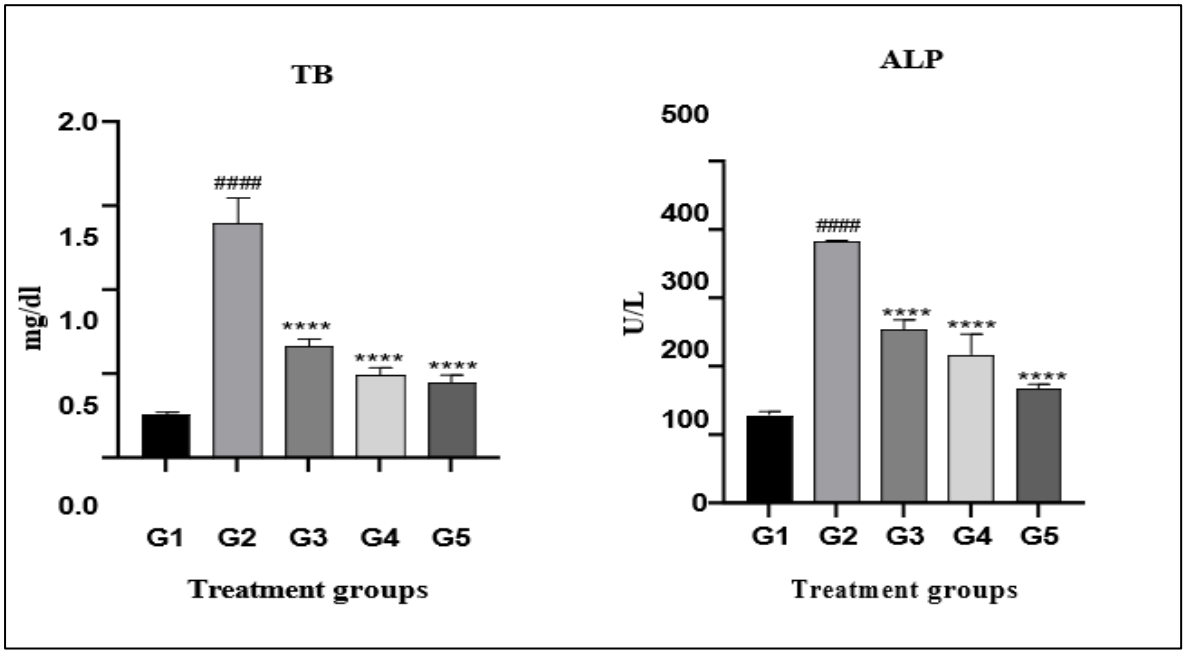
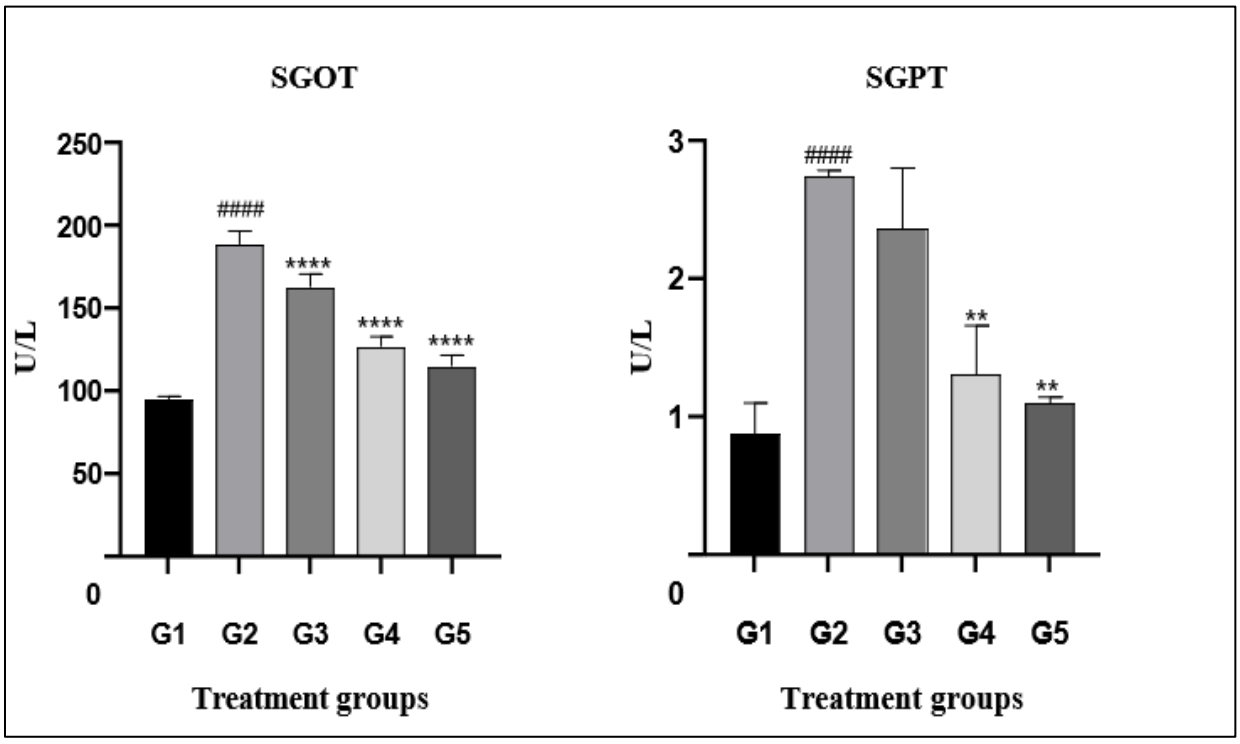


Fig no. 8- Effect of HAEIB on SGOT, SGPT, ALP, Total Protein and Total Bilirubin levels on Diclofenac induced Hepatotoxicity in rats.



Diclofenac administration (20 mg/kg, p.o.) significantly increased the serum SGOT and SGPT levels compared with the normal control group. The SGOT level increased from 94.49 ± 0.92 U/L to 188.8 ± 4.03 U/L, while the SGPT level increased from 0.88 ± 0.22 U/L to 2.738 ± 0.05 U/L in the diclofenac-treated group ($p<0.0001$). Treatment with HAEIB at doses of 200 mg/kg and 400 mg/kg reduced the elevated SGOT levels to 163.1 ± 3.50 U/L and 126.9 ± 2.50 U/L, respectively. Similarly, SGPT levels were reduced to 2.368 ± 0.43 U/L and 1.304 ± 0.36 U/L following treatment with HAEIB at 200 mg/kg and 400 mg/kg, respectively. Silymarin (50 mg/kg) also significantly reduced SGOT and SGPT levels to 115.0 ± 3.05 U/L and 1.102 ± 0.04 U/L, respectively, compared with the diclofenac-treated group (Table 6; figure 8).

iii) Effect on Antioxidant Parameters

Table no 7 - Effect of HAEIB on LPO, GSH and SOD levels in Diclofenac induced

Groups	Treatment	Glutathione (GSH)	Lipid peroxidation	Superoxide dismutase (SOD)

			(LPO)	
G1	Normal	109.5 ± 1.41	6.00 ± 0.11	16.56 ± 0.24
G2	Diclofenac alone (1 g/kg) p.o.	34.66 ± 0.61####	13.18 ± 0.40####	11.45 ± 0.24####
G3	Diclofenac (1 g/kg) p.o.+ HAEIB (200 mg//kg) p.o.	46.73 ± 0.98*	8.90 ± 0.12****	12.62 ± 0.24*
G4	Diclofenac (1 g/kg) p.o.+ HAEIB (400 mg/kg) p.o.	75.56 ± 3.75****	7.43 ± 0.21****	13.85 ± 0.35****
G5	Diclofenac (1 g/kg) p.o.+ Silymarin (50 mg/kg) p.o.	99.38 ± 4.59****	6.75 ± 0.06****	14.66 ± 0.10****

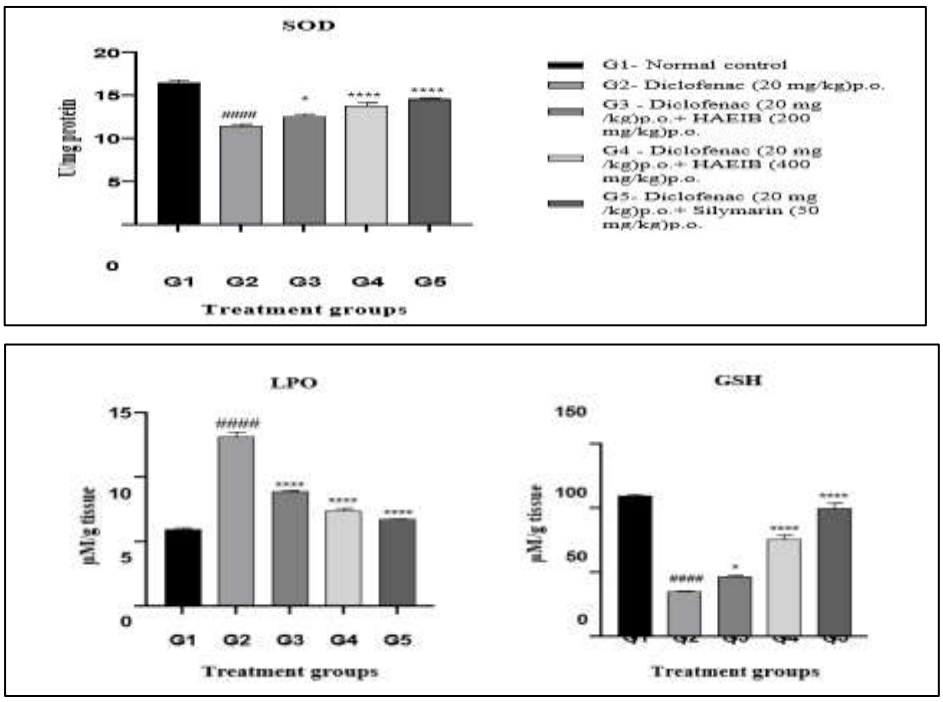
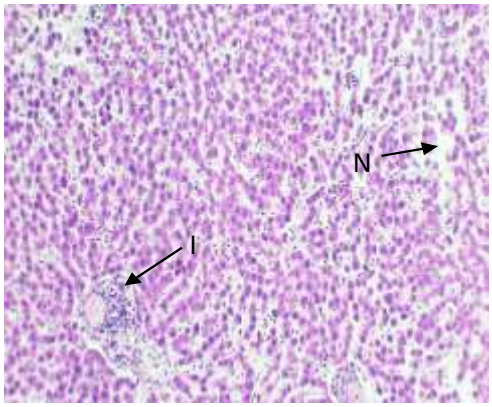


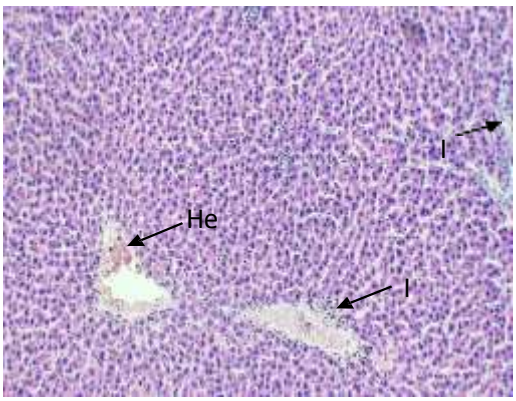
Fig no. 9 - Effect of HAEIB on LPO, GSH and SOD levels Diclofenac induced Hepatotoxicity in rats

Diclofenac administration (20 mg/kg, p.o.) significantly increased lipid peroxidation (LPO) levels from 6.00 ± 0.11 to 13.18 ± 0.40 ($p<0.0001$) and significantly decreased reduced glutathione (GSH) levels from 109.5 ± 1.415 to 34.66 ± 0.610 ($p<0.0001$) and superoxide dismutase (SOD) activity from 16.56 ± 0.244 to 11.45 ± 0.241 ($p<0.0001$) compared with the normal control group. Treatment with HAEIB at 200 mg/kg and 400 mg/kg significantly reduced LPO levels to 8.90 ± 0.12 and 7.43 ± 0.21, respectively, while significantly increasing GSH levels to 46.73 ± 0.98 and 75.56 ± 3.75, and SOD activity to 12.62 ± 0.24 and 13.85 ± 0.35, respectively. Silymarin (50 mg/kg) also significantly restored oxidative stress markers, reducing LPO to 6.757 ± 0.067 and increasing GSH and SOD levels to 99.38 ± 4.59 and 14.66 ± 0.10, respectively, compared with the diclofenac-treated group (table 7; fig 9).

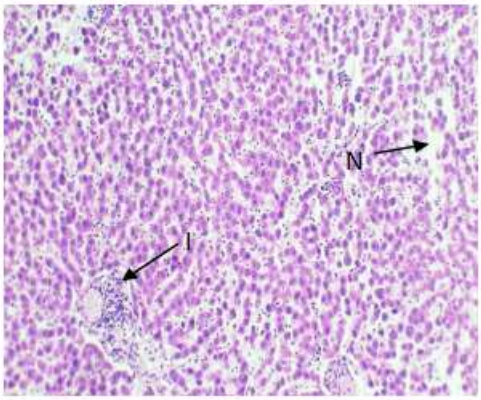
3.5 Histopathological Findings



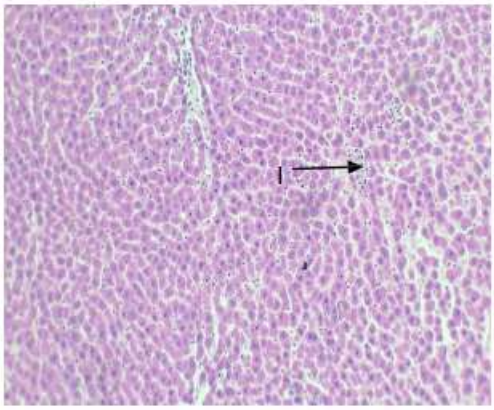
(a) Normal group



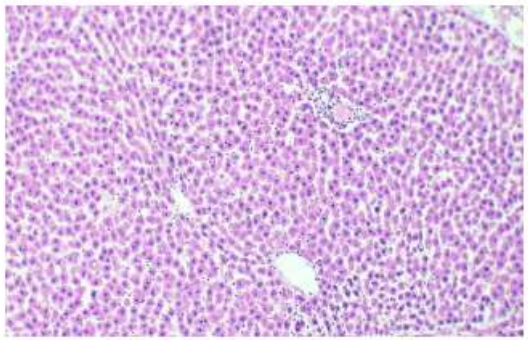
(b) Diclofenac 20 mg/kg b.w. alone



(c) Diclofenac 20 mg/kg b.w. + HAEIB (200mg/kg) b.w



(d) Diclofenac 20 mg/kg b.w.+ HAEIB (200mg/kg) b.w



(e) Diclofenac 20 mg/kg b.w.+ Silymarin (50 mg/kg) b.w.

Fig no 10 - Photomicrographs of rat liver (hematoxylin and eosin) (a) shows normal hepatic architecture; (b) haemorrhage, inflammation and necrosis; (C, D and E) show varying degrees of hepatic regeneration.

HC- Hepatocytes, PT – Portal triad, He – Haemorrhage, I – Infiltration of lymphocytes, N- Necrosis.
The histopathological examination of group a- livers showed a normal arrangement of the hepatocytes, with clearly visible nuclei, central vein and portal triad. The group b- Diclofenac 20 mg/kg b.w. intoxicated liver demonstrates infiltration of lymphocytes, the presence of haemorrhage and necrosis. Group c- Liver section of rats treated with Diclofenac 20 mg/kg b.w. and 200 mg/kg bw of HAEIB showed less inflammatory cells and reduced necrosis. Group d- Liver section of rats treated Diclofenac 20 mg/kg b.w. and 400 mg/kg b.w. of HAEIB showed minimal inflammatory cellular infiltration, reduced necrosis, regeneration of hepatocytes was also observed and almost near normal liver architecture Group e- Liver section of rats treated with Diclofenac 20 mg/kg b.w. and 50 mg/kg b.w of silymarin showed normal liver tissue.

4. DISCUSSION

The liver is highly susceptible to damage caused by xenobiotics and oxidative stress due to its central role in drug metabolism. Diclofenac-induced hepatotoxicity is characterized by elevated serum liver enzymes (SGOT, SGPT, ALP, and total bilirubin), decreased total protein, increased lipid peroxidation (LPO), depletion of endogenous antioxidants (GSH and SOD), and histopathological alterations, reflecting hepatocellular injury and impaired liver function.

In the present study, the hydroalcoholic extract of Impatiens balsamina leaves (HAEIB) significantly attenuated diclofenac-induced liver damage. Treatment with HAEIB restored serum biochemical markers toward normal levels, reduced oxidative stress by decreasing LPO, enhanced antioxidant defenses through increased GSH and SOD levels, and improved liver histoarchitecture. The hepatoprotective effect was dose-dependent, with the 400 mg/kg dose showing greater efficacy and effects comparable to those of the standard drug, silymarin.

The hepatoprotective activity of HAEIB may be attributed to its rich phytochemical composition, including flavonoids, phenolic compounds, tannins, glycosides, and terpenoids. These bioactive constituents are known for their antioxidant and free radical scavenging properties, which help neutralize reactive oxygen species, reduce lipid peroxidation, preserve hepatocyte membrane integrity, and enhance endogenous antioxidant defense mechanisms. The quantitative phytochemical analysis demonstrating the presence of flavonoids, phenolics, and tannins further supports the observed hepatoprotective activity.

Overall, the findings indicate that HAEIB possesses significant hepatoprotective and antioxidant potential against diclofenac-induced hepatotoxicity. However, further studies are required to isolate the active constituents and elucidate the precise molecular mechanisms responsible for its hepatoprotective effects.

5. CONCLUSION

The present study demonstrated that the hydroalcoholic extract of Impatiens balsamina leaves (HAEIB) possesses significant hepatoprotective activity against diclofenac-induced hepatotoxicity in Wistar rats. HAEIB treatment significantly restored altered biochemical markers, reduced oxidative stress by decreasing lipid peroxidation (LPO) and enhancing antioxidant enzymes (GSH and SOD), and improved liver architecture. The hepatoprotective effect was dose-dependent, with the 400 mg/kg dose showing greater efficacy and results comparable to the standard drug, silymarin. The observed activity may be attributed to the presence of bioactive phytoconstituents, particularly flavonoids, phenolics, and tannins, which possess antioxidant properties. These findings suggest that HAEIB has promising potential as a natural hepatoprotective agent. However, further studies are required to isolate the active constituents and elucidate the precise molecular mechanisms underlying its hepatoprotective effects.

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