RESEARCH PAPER

EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF *IMPATIENS BALSAMINA LINN*. LEAVES EXTRACT IN WISTAR RAT

Kirti Arya*, Dr. Satpute Kranti, Dr. Wadulkar Raghunath
Department of Pharmacology, Dayanand Education Society's, Dayanand College of Pharmacy, Latur, Maharashtra 413512 India

***Corresponding Author,**
Kirti Arya,
Department of Pharmacology,
Dayanand Education Society's,
Dayanand College of Pharmacy,
Latur, Maharashtra 413512 India
Email: kirtiarya098@gmail.com

ABSTRACT:
The present study investigates the hepatoprotective potential of ethanolic leaf extract of *Impatiens balsamina* Linn. in opposition to carbon tetrachloride (CCl_4) - caused Wistar rats' livers to become poisonous. DPPH assay-based in vitro antioxidant screening and in vivo evaluation through biochemical and histopathological analysis. CCl_4 administration significantly altered liver function biomarkers such as bilirubin, protein levels and liver enzymes (ALT, AST, and ALP). These metabolic changes were dramatically and dose-dependently reversed by treatment with the plant extract. showing results comparable to silymarin. Histopathological observations further confirmed hepatic protection, with restoration of normal liver architecture in treated groups. These findings suggest that *Impatiens balsamina* Linn. Possesses significant hepatoprotective properties, likely attributed to its phytoconstituents including flavonoids and phenolic compounds.

Keywords:
Impatiens balsamina, hepatoprotective, carbon tetrachloride, liver biomarkers, DPPH assay, silymarin, antioxidant.

How to cite this article: Arya K, Kranti S, Raghunath W. Evaluation of Hepatoprotective Activity of Impatiens balsamina Linn. Leaves Extract in Wistar Rat. Int J Drug Deliv Technol. 2026;16(72s): 971-984. DOI: 10.25258/ijddt.16.72s.108

INTRODUCTION:

The largest internal organ in the body is the liver. This becomes apparent into the last weeks of development in a growing embryo. It has a reddish-brown hue. It has four lobes: right, left, caudate, and quadrate, and is positioned on the right side of the abdomen, just beneath the vertebral column. A liver typically weighs between 1400 and 1600 grams. This acts as the main metabolic organ and a complicated gland that carries out a number of interconnected tasks that are vital to life. The bile duct connects with the liver's more than 300 billion specialized cells together.[1] From a vein known as the portal vein, the liver collects around 1½ litters of nutrient-rich blood per minute from the small intestinal tract. This enables the liver to regulate the amount of nutrients that are supplied to the rest of the body while eliminating and eliminating toxic and useless molecules.[2] an essential organ, the liver has a role in metabolism. Detoxifying of poisons and medicinal substances, as well as the bio-regulation of proteins, lipids, carbohydrates, amino acids, blood coagulation, and immunomodulation. Excessive exposure to xenobiotics, alcohol, chemotherapeutic drugs, and infectious agents typically results in loss of its ability to operate. Viral hepatitis can progress to persistent hepatitis, which, if ignored, causes fibrosis or malignant tumors, depending upon the extent of the liver cell damage.[3]

Plant profile:

Often referred to as "garden balsam" or "rose balsam," *Impatiens balsamina* is a perennial herb that is a member of the Balsaminaceae family. This plant is indigenous to India in southern Asia. Traditional Ayurvedic, Unani, and Siddha practices use *Impatiens balsamina* to treat a variety of illnesses and physiological abnormalities, including jaundice, corns, snake bites, and more.[4] Numerous beneficial substances, including naphthoquinones, phenolic compounds, flavonoids, tannin, Saponin and steroids, were found by phytochemical investigations and were absence of alkaloid. Distinct plant parts, such as leaves, stem juice, and flowers, have distinct applications. Numerous therapeutic actions, including antimicrobial, antifungal, analgesic, anti-inflammatory, antioxidant, and antipruritic properties, have been described for Impatiens balsamina. Ingrown nails, abscesses,

thorn or glass-puncture wounds, and persistent ulcers brought on by allergic reactions have all historically been treated using *I. balsamina*.[5]

Several medicinal uses, such as antitumor, antifungal, anti-fibrosis, antidiabetic, antinociceptive, antimicrobial, wound healing, anti-arthritic, antipruritic, anti-dermatic, antihelminthic, antihistamine, and anti-inflammatory effects, have been reported for Impatiens balsamina Linn. Since the majority of practitioners in Indian medical systems create and administer their own medications, these calls for appropriate recording and study.[6]

MATERIALS AND METHODS:

Extraction Procedure:

Collection and Authentication: Fresh leaves of *Impatiens Balsamina* Linn were collected in Latur, India in December-January (2024-2025) and was authenticated by Department of Botany at Dayanand Science College, Latur.

Preparation of Extracts: The leaves of *Impatiens Balsamina* Linn dried under shade dry 50g of dry powder extracted for 8 hours with ethanol solvent through Soxhlet apparatus. The powder is extracted until the cycles are completed and become colourless. Then the Soxhlet used for recovery of solvent. Whatman No. 1 filter paper was used to filter the gathered solutions. The extract was chilled and evaporated once the extraction process was finished. The extract was kept in an airtight container. The extract yield percentage was computed.

$$\%\ \boldsymbol{Yield} = \frac{\boldsymbol{Weight\ of\ Extract\ Obtained}}{\boldsymbol{Weight\ of\ Starting\ Material}} \times \mathbf{100}$$

IN-VITRO SCREENING MODEL:

DPPH ASSAY: FREE-RADICAL SCAVENGING ACTIVITY

The DPPH method was used to assess the ethanolic extract of Impatiens balsamina linn's ability to scavenge free radicals. It was determined by measuring the drop in absorbance at 517 nm of a DPPH solution in methanol caused by the sample.

1.3 mg/ml of DPPH in methanol was made into a stock solution. Prepare a 10 mg/10 ml ethanolic extract concentration for the sample. 1ml of this solution was transferred to test tubes, and the remaining were diluted using the same solvent. This is a stock solution. 0.10, 0.15, 0.25, 0.50, and 0.60 ml of the stock solution were extracted and placed in separate test tubes. Their respective concentrations were 10, 15, 25, and 50 micrograms per milliliter,, that included the same amount of ethanolic extracts. Ascorbic acid as a reference and control sample with the same volume but no extract were prepared beforehand. The blank was 95% methanol.

The following formula was employed to determine the proportion of the DPPH free radical scavenged.[7]

% inhibition = {(A control – A sample) / (A control)} × 100

Absorbance of DPPH alone serves as a control.

A sample consists of various extract concentrations and DPPH absorbance.

Plotting a graph of concentration versus percentage inhibition yielded an equation of line that was used to compute IC50.[7]

Ethical Approval:

The study will be was out in compliance with moral standards and authorized by the Institutional Animal Ethics Committee at **DES Dayanand College of Pharmacy, Latur & IAEC Protocol No: [DCOP/IAEC/2024-25/15].** Care will be taken to minimize animal suffering and adhere to the principles of animal welfare.

Experimental Animals:

All the experiments were carried out in Wistar rats (180 -200gm). Animals were procured from **Crystal Biological Solutions, Dglobal building, Sai Park, Uruli Devachi Pune-412308 Reg No: 2030/PO/RcBiBt/S/18/CPCSEA Maharashtra**. The animals were kept in polypropylene cages, approximately six per cage, with a 12-hour light/dark cycle, regulated humidity (30–70%) and temperature (22±3°C), unrestricted access to water, and the specific diet**.**

Table 1: Animal Requirement

Species and strain	Weight of animals	Sex	No. of Animals
Wistar rat	180-200 gm	Either	30 (N=6)

IN-VIVO SCREENING MODEL:

Hepatoprotective activity study:

Carbontetrachoride induced hepatotoxicity model used for evaluation and comparison of hepatoprotective activity of *Impatiens Balsamina* Linn.

Thirty rats were divided randomly into 5 groups, each compromise 6 animal. Before being treated with carbon tetrachloride, the animals were fasted for a full day.

Group I: (Normal control group): Receives normal solution of saline 5ml/kg body weight by orally

- **Group IInd (Inducer group**) received no medication therapy and was kept as a carbon tetrachloride control.
- **Group IIIrd (standard group):** Receives silymarin 100mg/kg of body weight orally which served as standard group.
- **Group IVth, Group Vth (test group)** animals are treated with 200 mg/kg and 400mg/kg ethanolic extract, respectively, orally.
- **Group III to group VIth** receives carbon tetrachloride diluted with olive oil (1:1) at

dose 1ml/kg for the second and third days in a row. From the first to the fifth day, the vehicle or medication treatment was administered orally, with concomitant administration of CCL4 on the second and third days. The rats were given a regular meal and unlimited access to water over the course of the medication therapy.

- on 6th day animal anesthetized by using the diethyl ether anaesthetic agent, and a blood sample was collected from retro orbital route for biochemical parameter estimation and then sacrificed followed by cervical dislocation The liver was separated and kept in a 10% formalin solution..[8,9]

HISTOPATHOLOGY PROCESS:

The animal tissue sample was isolated and put in a 10% neutral buffer formalin solution and specimen sample was send to PreclinBio solution, Pune for Histopathology study.

RESULT:

Results of Phytochemical Screening.

The extract was examined to check for the presence of several chemical groups of compounds as per the techniques described. Preliminary phytochemicals screening shows the presences of relatively the existence of moderately phytochemical flavonoids is revealed by preliminary phytochemical screening.

Body Weight:

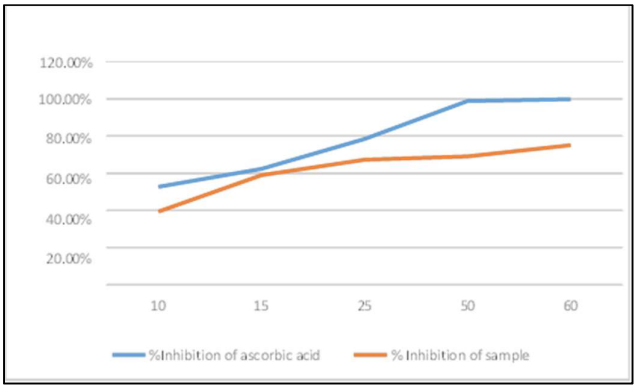
Table No 2: Table of body weight of animal.

Group	Initial Body Weight	Body Weight on 5th Day
Control	203.13 ± 0.46	204.78 ± 0.45
Inducing	178.8 ± 0.47	170.65 ± 0.46
Standard	182.25 ±0.46	192.46 ± 0.46
Test I (200 mg)	161 ± 0.47	180 ± 0.45
Test II (400 mg)	163 ± 0.47	190 ± 0.46

Result of DPPH.

Table No. 3: Results of DPPH

Concentration (µg/ml)	% Ascorbic Acid Inhibition	% Extract of Inhibition
10	52.74%	39.39%
15	62.33%	59.09%
25	78.57%	67.42%
50	98.79%	69.09%
60	99.86%	75.15%



Graph No. 1: % inhibition of Sample and Ascorbic acid by DPPH assay

Ascorbic acid has greater antioxidant activity at all concentrations, according to the graph, but the test sample also exhibits a notable and growing inhibitory effect, reaching up to 75.15% at 60 µg/ml.

Liver Biomarker Result:

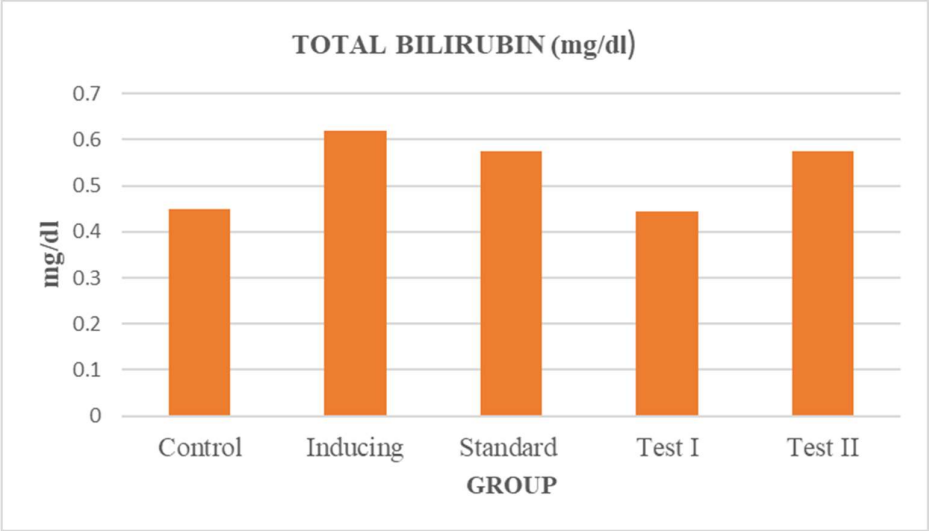
Total Bilirubin Level:

Table No. 4: Total Bilirubin Level

Groups	Total Bilirubin (mg/dl)
Control(5ml/kg)normal saline	0.45± 0.05
Inducer(1ml/kg) CCL4	0.62± 0.05
Standard(100mg/kg)silymarin+CCL4	0.57 ± 0.07
Test I (200 mg/kg) EEIBL+CCL4	0.43± 0.07
Test II (400 mg/kg) EEIBL+CCL4	0.57 ± 0.06

The values are presented as mean ± SEM, with n = 6 for each group. One-way ANOVA and the Tukey test were used for statistical analysis. Show the CCL4 inducer comparison between the silymarin group and EEIBL ($p<0.05$).

Graph No. 2: Level of Total Bilirubin

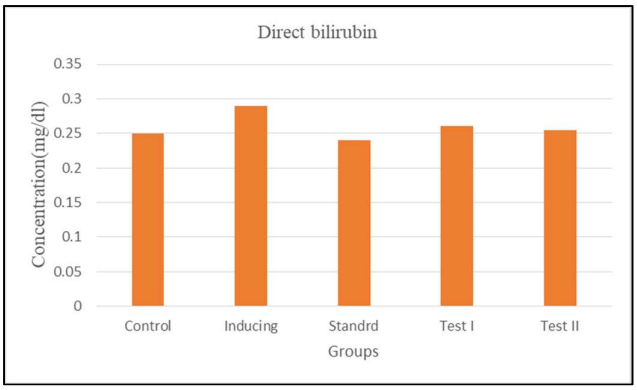


Level of Direct Bilirubin:

Table No. 5: Direct Bilirubin Level

Groups	Level of Direct bilirubin(mg/dl)
Control(5ml/kg)normal saline	0.25 ± 0.03
Inducer (1ml/kg) CCL4	0.29 ± 0.03
Standard(100mg/kg)silymarin+CCL4	0.24 ± 0.02
Test I (200mg/kg) EEIBL+CCL4	0.255 ± 0.031
Test II (400mg/kg)EEIBL+CCL4	0.250 ± 0.078

The values are presented as mean ± SEM, with n = 6 for each group. One-way ANOVA and the Tukey test were used for statistical analysis. Show the CCL4 inducer comparison between the silymarin group and EEIBL ($p<0.05$).



Graph No. 3: Level of Direct Bilirubin

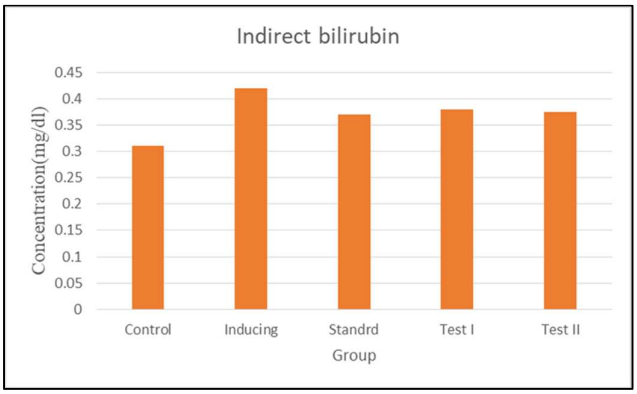
Level of Indirect Bilirubin:

Table No. 6: Indirect Bilirubin Level.

Groups	Indirect bilirubin

Control(5ml/kg)normal saline	0.31±0.04
Inducer(1ml/kg) CCL4	0.42± 0.05
Standard(100mg/kg)silymarin+CCL4	0.37±0.03
Test I (200 mg/kg) EEIBL+CCL4	0.38± 0.12
Test II (400 mg/kg) EEIBL+CCL4	0.375± 0.032

The values are presented as mean ± SEM, with n = 6 for each group. One-way ANOVA and the Tukey test were used for statistical analysis. Show the CCL4 inducer comparison between the silymarin group and EEIBL ($p<0.05$).



Graph No. 4: Level of Indirect Bilirubin

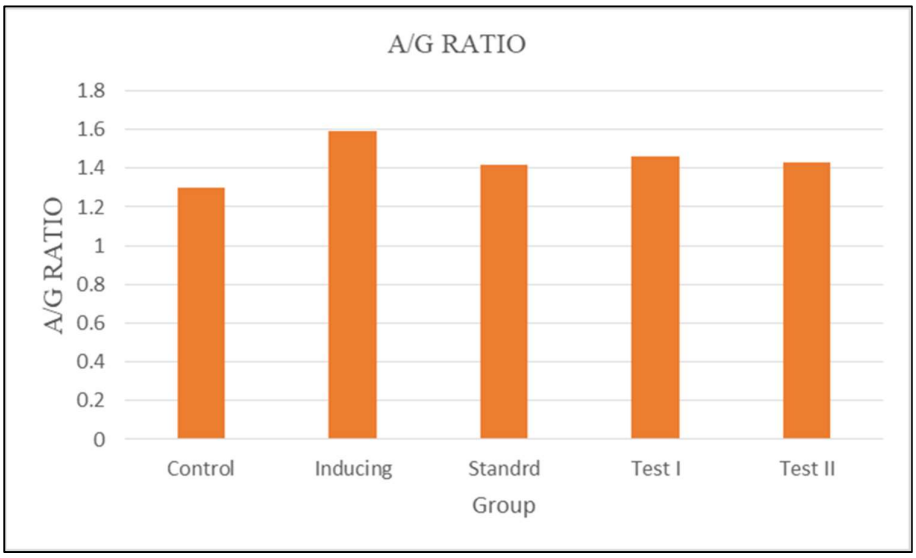
A/G RATIO:

Table No. 7: A/G RATIO

Groups	**A/G RATIO**
Control(5ml/kg)normal saline	1.30 ± 0.03
Inducer(1ml/kg) CCL4	1.59 ± 0.3
Standard(100mg/kg)silymarin+CCL4	1.42 ± 0.12
Test I (200 mg/kg) EEIBL+CCL4	1.46 ± 0.24
Test II (400mg/kg) EEIBL+CCL4	1.435 ± 0.27

The values are presented as mean ± SEM, with n = 6 for each group. One-way ANOVA and the Tukey test were used for statistical analysis. Show the CCL4 inducer comparison between the silymarin group and EEIBL ($p<0.05$).

Graph No. 5: A/G RATIO

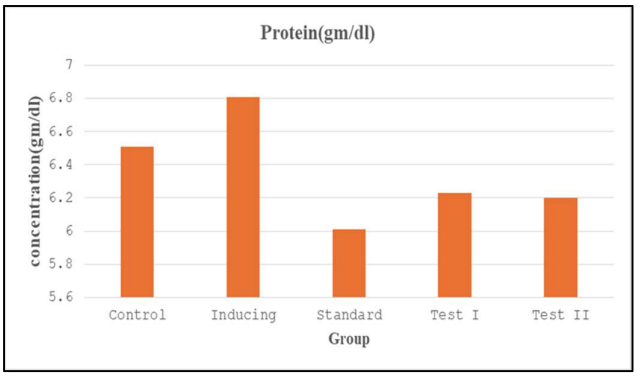


Protein:

Table No. 8: Protein level

Group	Protein
Control(5ml/kg)normal saline	6.51 ± 0.10
Inducer(1ml/kg) CCL4	6.81± 0.29
Standard(100mg/kg)silymarin+CCL4	6.01± 0.20
Test I (200 mg/kg) EEIBL+CCL4	6.23± 0.25
Test II (400mg/kg) EEIBL+CCL4	6.20 ±0.13

The values are presented as mean ± SEM, with n = 6 for each group. One-way ANOVA and the Tukey test were used for statistical analysis. Show the CCL4 inducer comparison between the silymarin group and EEIBL ($p<0.05$).



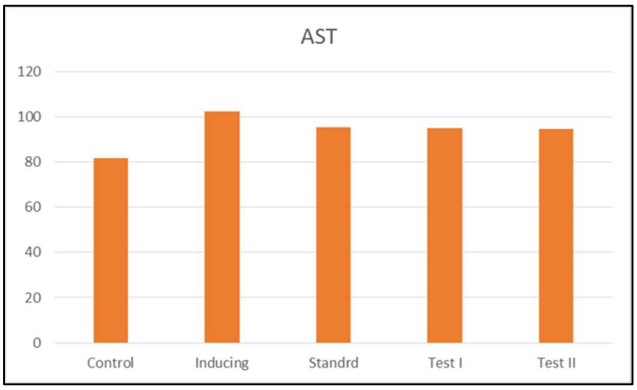
Graph No. 6: Protein Level

AST

Table No. 9: AST

Group	AST
Control(5ml/kg)normal saline	81.97 ±7.69
Inducer(1ml/kg) CCL4	102.40± 8.77
Standard(100mg/kg)silymarin+CCL4	95.35 ± 7.79
Test I (200 mg/kg) EEIBL+CCL4	95.20± 5.99
Test II (400 mg/kg) EEIBL+CCL4	94.87 ± 4.61

The values are presented as mean ± SEM, with n = 6 for each group. One-way ANOVA and the Tukey test were used for statistical analysis. Show the CCL4 inducer comparison between the silymarin group and EEIBL ($p<0.05$).



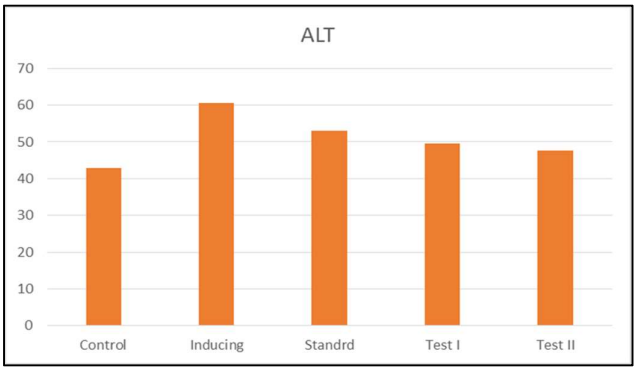
Graph No. 7: AST

ALT Level

Table No. 10: ALT Level

Groups	ALT
Control(5ml/kg)normal saline	42.96±4.00
Inducer(1ml/kg) CCL4	60.59± 5.06
Standard(100mg/kg)silymarin+CCL4	52.96 ± 2.09
Test I (200 mg/kg) EEIBL+CCL4	49.62± 1.463
Test II (400 mg/kg)EEIBL+CCL4	47.68± 6.6

The values are presented as mean ± SEM, with n = 6 for each group. One-way ANOVA and the Tukey test were used for statistical analysis. Show the CCL4 inducer comparison between the silymarin group and EEIBL ($p<0.05$).



Graph No. 8: ALT Level

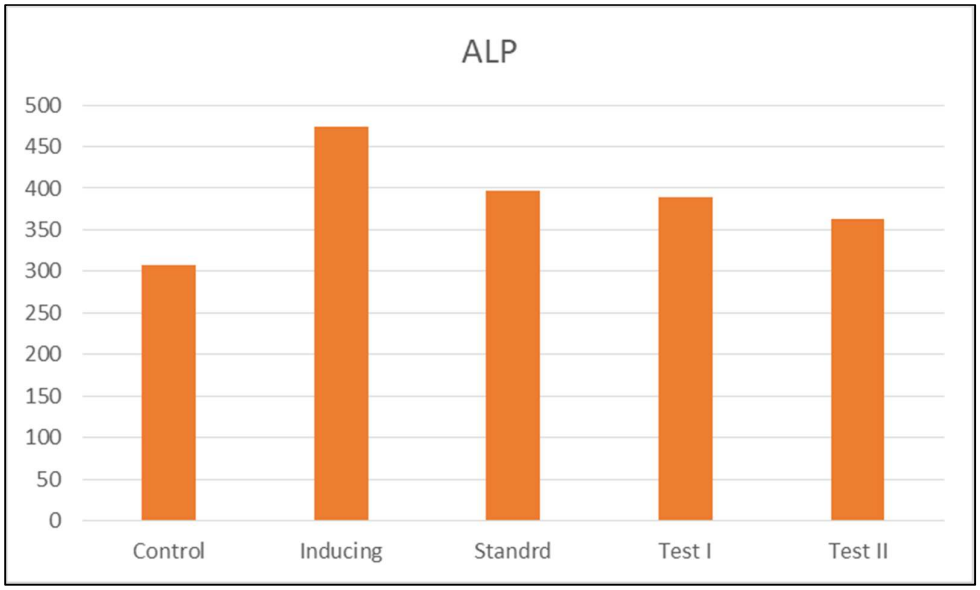
Alkaline Phosphate

Table No. 11: Alkaline Phosphate

Group	**ALP**
Control(5ml/kg)normal saline	307.43±36.44
Inducer(1ml/kg) CCL4	474.46± 51.77
Standard(100mg/kg)silymarin+CCL4	396.75±27.78
Test I (200 mg/kg)EEIBL+CCL4	388.51 ±25.37
Test II (400 mg/kg)EEIBL+CCL4	363.32 ± 23.88

The values are presented as mean ± SEM, with n = 6 for each group. One-way ANOVA and the Tukey test were used for statistical analysis. Show the CCL4 inducer comparison between the silymarin group and EEIBL ($p<0.05$).

Graph No. 9: Alkaline Phosphate



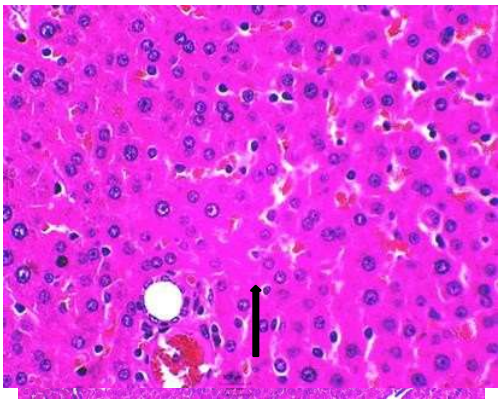
Over all data of liver biomarker:

Parameter	**Control(5ml/kg) Normal saline**	**Inducer (1ml/kg) CCL4**	**Standard (Silymarin 100 mg/kg) +CCL4**	**Test I (200 mg/kg EEIBL+CCL4**	**Test II (400 mg/kg) EEIBL+CCL4**
Total Bilirubin (mg/dL)	0.45 ± 0.05	0.62 ± 0.05	0.57 ± 0.07	0.43 ± 0.07	0.57 ± 0.06
Direct Bilirubin (mg/dL)	0.25 ± 0.03	0.29 ± 0.03	0.24 ± 0.02	0.255 ± 0.031	0.250 ± 0.078
Indirect Bilirubin (mg/dL)	0.31 ± 0.04	0.42 ± 0.05	0.37 ± 0.03	0.38 ± 0.12	0.375 ± 0.032
ALT (U/L)	42.96 ± 4.00	60.59 ± 5.06	52.96 ± 2.09	49.62 ± 1.463	47.68 ± 6.6
AST (U/L)	81.97 ± 7.69	102.40 ± 8.77	95.35 ± 7.79	95.20 ± 5.99	94.87 ± 4.61
ALP (U/L)	307.43 ± 36.44	474.46 ± 51.77	396.75 ± 27.78	388.51 ± 25.37	363.32 ± 23.88
Total Protein (g/dL)	6.51 ± 0.10	6.81 ± 0.29	6.01 ± 0.20	6.23 ± 0.25	6.20 ± 0.13
A/G Ratio	1.30 ± 0.03	1.59 ± 0.3	1.42 ± 0.12	1.46 ± 0.24	1.435 ± 0.27

***Note**: n = 6 rats per group; values are given as mean ± SEM. Tukey's test (p < 0.05) was used after one-way ANOVA to establish statistical significance.*

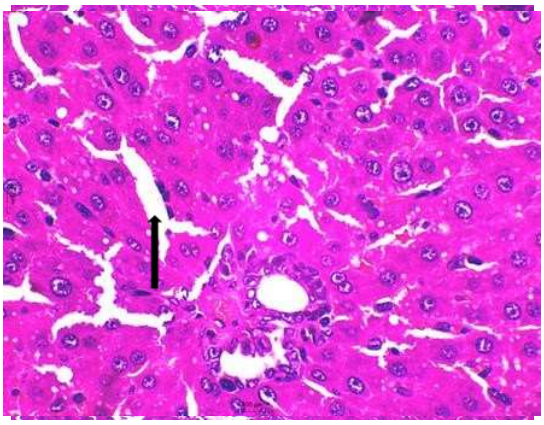
HISTOPATHOLOGY RESULTS:

Normal Control group Animal (10 X) **Normal Control group Animal (40X)**



HISTOPATHOLOGICAL OBSERTVATION: Normal hepatocytes are clearly sinusoidal and grouped in cords. No abnormality detected.

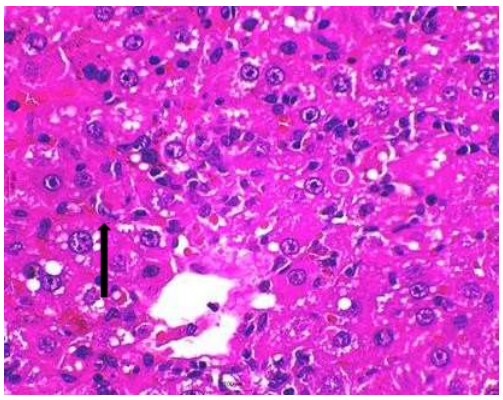
CCl4 Induced group Animal (10X) **CCl4 Induced group Animal (40X)**



HISTOPATHOLOGICAL OBSERTVATION: Hepatic cells exhibiting diffuse alterations, localized necrotic cell death, and ballooning degeneration. Abnormality detected.

Standard group (silymarin) Animal

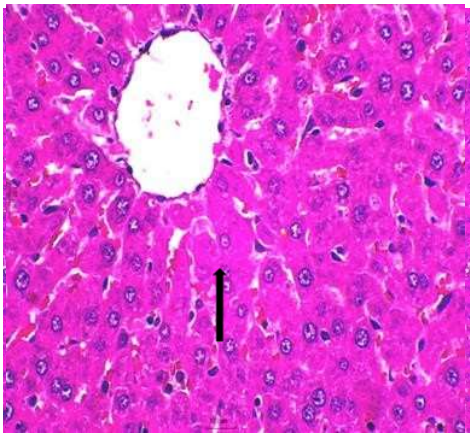
Standard group (silymarin) Animal (10X) (40X)



HISTOPATHOLOGICAL OBSERTVATION: Normal hepatocytes are clearly sinusoidal and grouped in cords. No abnormality detected.

Test Group I Animal (10X)

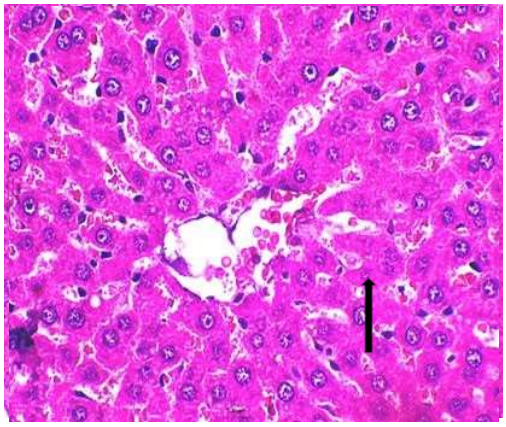
Test Group I Animal (40X)



HISTOPATHOLOGICAL OBSERTVATION: Normal hepatocytes are clearly sinusoidal and grouped in cords. No abnormality detected.

Test Group II Animal (10X) **Test Group II Animal (40X)**

HISTOPATHOLOGICAL OBSERTVATION: Normal hepatocytes are clearly sinusoidal and grouped in cords.



No abnormality detected.

The liver tissues were examined histopathologically to compare microscopic changes among different groups: the normal group showed regular hepatic architecture; the inducer (CCl_4-treated) group exhibited, diffuse alterations, localized necrotic cell death, and ballooning degeneration; the standard group (treated with a known hepatoprotective drug) displayed marked improvement with restored liver structure; and the test group (treated with plant extract) showed dose-dependent recovery, indicating potential hepatoprotective effects.

DISCUSSION:

This study evaluated the Impatiens balsamina Linn's ethanolic leaf extract's hepatoprotective properties against carbon tetrachloride (CCl4)-induced in wistar rats. The experiment employed multiple biochemical, antioxidant, and Histopathological parameters to determine the extract's efficacy.

Biochemical analysis demonstrated that CCl_4 significantly increased levels of total, direct, and indirect bilirubin, indicating liver dysfunction.

Treatment with the extract, particularly at the higher dose, normalized these parameters. Similarly, elevated levels of AST, ALT, and ALP liver enzymes in the CCl4 group were substantially reduced in extract-treated groups, indicating stabilization of hepatocyte membranes and reduced enzyme leakage. A/G ratio and total protein also showed considerable improvement with extract treatment, reflecting restoration of liver synthetic function.

- Histopathological examination corroborated the biochemical findings. The CCl4 group exhibited ballooning degeneration, necrosis, and hepatic damage.
- In contrast, the liver architecture of treated groups remained intact, with normal hepatocyte arrangement, confirming the extract's protective function.
- Stastical analysis done by using A one-way ANOVA reveals a significant value ($p<0.05$).

CONCLUSION:

- The preclinical evaluation of *Impatiens balsamina* demonstrated significant hepatoprotective effect in Wistar rats, as evidenced by improved biochemical markers and supportive histopathological findings, in contrast to silymarin, a standard hepatoprotective medication.
- These results indicate Impatiens balsamina's potential as a natural hepatoprotective agent. However, further investigations including detailed pharmacological, toxicological, and clinical studies are required to establish its safety and efficacy for clinical use.

CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest.

REFERENCES:

1. Waugh A, Grant A. Ross & Wilson Anatomy and Physiology in Health and Illness. 14th ed. Edinburgh: Elsevier; 2022.
2. Hall JE. Guyton and Hall Textbook of Medical Physiology. 14th ed. Philadelphia: Elsevier; 2021.
3. Kumar V, Abbas AK, Aster JC. Robbins and Cotran Pathologic Basis of Disease. 10th ed. Philadelphia: Elsevier; 2021.
4. Sharma PC, Yelne MB, Dennis TJ. Database on Medicinal Plants Used in Ayurveda. Vol. 2. New Delhi: Central Council for Research in Ayurveda and Siddha (CCRAS), Ministry of AYUSH, Government of India; 2005.
5. Yu SX, Kim YH, Lee SH, Kim HJ. Chemical constituents and biological activities of Impatiens balsamina L.: A review. J Med Plants Res. 2011;5(11):2400-5.
6. Liu Y, Ali MY, Choi RJ, Sun Z, Kim YS, Lee SH, et al. Phytochemicals and pharmacological activities of Impatiens balsamina: A review.

Plants (Basel). 2020;9(10):1389. doi:10.3390/plants9101389.
7. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT Food Sci Technol. 1995;28(1):25-30.
8. Recknagel RO, Glende EA Jr, Dolak JA, Waller RL. Mechanisms of carbon tetrachloride toxicity. Pharmacol Ther. 1989;43(1):139-54.
9. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 56th ed. Pune: Nirali Prakashan; 2021.
10. Mahli A, Koch A, Czech B, Peterburs P, Lechner A, Haunschild J, Müller M, et al. Hepatoprotective effect of oral application of a silymarin extract in carbon tetrachloride-induced hepatotoxicity in rats. Clin Phytosci. 2015;1:5. doi:10.1186/s40816-015-0006-6.