



GCMS analysis and Hepatoprotective effect of *Impatiens henslowiana* on Galactosamine -Induced Hepatotoxicity in HepG2 Cells

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KEYWORDS

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ABSTRACT:

Introduction: The liver is a vital organ in the human body, performs numerous essential functions, rendering it vulnerable to various liver diseases. Damage to the liver typically involves cell death, increased oxidative stress, and decreased levels of glutathione, a crucial antioxidant. Despite advancements in medical science, liver diseases continue to pose a significant global health challenge. Conventional drugs used to treat liver diseases often fall short and may have adverse effects. Therefore, there is a growing interest in exploring alternative treatments, particularly those derived from traditional medicinal plants.

Objectives: This study aimed to validate the hepatoprotective activity of extracts from *Impatiens henslowiana* against Galactosamine-induced hepatotoxicity in HepG2 cells.

Methods: The cells were exposed to different extracts at concentrations ranging from 1.56 µg/ml to 25 µg/ml in combination with Galactosamine (0.1%) for 24 hours. The hepatoprotective effects were assessed through various assays, including cell proliferation and the oxidative stress mechanism.

Results: The anti-proliferative effect was enhanced in the presence of all plant extracts at all tested concentrations. The ethanol extract-treated HepG2 cells exhibited a 64.45% reduction in the Galactosamine-induced group. Galactosamine led to a 198.25% increase in MDA level, which was attenuated by the ethanol extract at 169.34% at 6.25 µg/ml. GSH content in cells decreased by 52.25% with Galactosamine, but the plant extract increased GSH levels by 64.38%. Additionally, Galactosamine induced a 34.26% decrease in SOD levels, but this necrotic effect was diminished by the treatment group to 51.62%.

Conclusions: In conclusion, extracts from *Impatiens henslowiana* demonstrated a preventive effect on HepG2 cell injury induced by Galactosamine treatment for 24 hours, and the hepatoprotective activity of the ethanol extract was comparable to that of the standard drug silymarin.

1. Introduction

The liver plays a critical role in metabolism, detoxification, and excretion processes, making it susceptible to xenobiotics and prone to toxicity-induced morphological and functional changes [1,2,3]. Given its central involvement in various functions, the liver is especially vulnerable to reactive oxygen species (ROS), which can impair the cell's defence mechanisms,

initiating oxidative stress. Consequent cellular damage may lead to inflammation and fibrosis, and in more severe instances, it can contribute to the development of cancer and necrosis. Galactosamine is commonly used to induce oxidative stress and liver injury, playing a pivotal role in assessing the therapeutic potential of drugs and dietary antioxidants in experiments involving HepG2 cells [4,5]. Administration of single dose of



Galactosamine results in dose dependent hepatic damage resembling viral hepatitis, with focal necrosis and periportal inflammation. It induces hepatitis by hindering the synthesis of RNA and protein via reduction in cellular UTP uptake that tips to the hepatic parenchyma necrosis [6,7]. Furthermore, the xenobiotic inflicts additional damage to liver cell membranes and organelles, leading to swelling, hepatocyte necrosis, and the release of cytosolic enzymes, including aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH), into the serum, ultimately resulting in cell death.

The utilization of medicinal plants and their derived products is widespread, especially in developing countries, and is experiencing a significant surge. This phenomenon is largely ascribed to the impact of empirical knowledge passed down through generations and the growing public interest in natural therapies. Many individuals rely on these products, assuming their safety due to their natural origin. However, plants contain a diverse array of compounds, some of which may pose a potential risk for inducing liver damage. Unfortunately, there is limited information available regarding the toxicological and pharmacological profiles of these compounds. Additionally, numerous cases of intoxication remain underreported. Hence, it is imperative to scrutinize the composition of medicinal plants to identify compounds that may harbour hepatotoxic potential.

On the contrary, medicinal plants may harbor beneficial compounds that contribute to the exploration of novel medicines. Scientific investigations have revealed that secondary metabolites such as polyphenols, anthraquinones, terpenes, and sulfuraphane possess the ability to activate the antioxidant defence system in hepatocytes, with Nrf2 playing a central role. This activation is crucial in mitigating damage caused by oxidative stress and offering protection to the liver [8].

Impatiens henslowiana, belonging to the Balsaminaceae family, is a flowering plant native to the Indian states of Kerala and Tamil Nadu, as well as Sri Lanka. This plant is characterized as a sizable shrub that can grow either terrestrially or epiphytically. The genus *Impatiens*, boasting over 1,000 species, is widely distributed in the tropical and subtropical regions of the Old World, including the northern temperate regions.

This diverse genus is recognized for having five distinct diversity hotspots: tropical Africa, Madagascar, Southern India and Sri Lanka, the Eastern Himalaya, and Southeast Asia [9,10].

The utilization of *Impatiens henslowiana* as a single-plant remedy for addressing liver ailments among tribal communities is presented in this report. This ethnomedical information holds the potential to guide the development of valuable drugs aimed at treating liver diseases. The comprehensive utilization of the entire *Impatiens henslowiana* plant is based on its traditional recognition for possessing high hepatoprotective properties, especially in the treatment of jaundice [11].

The ethanol and water extract obtained from *Impatiens henslowiana* exhibited significant anti-inflammatory activity. Additionally, the leaf and root extract derived from the *Impatiens henslowiana* demonstrated the most potent antimicrobial activity [12].

2. Objectives

Therefore, the current study was conducted to assess the hepatoprotective effects of the plant extract derived from *Impatiens henslowiana* in a HepG2 cell line subjected to injury induced by Galactosamine.

3. Methods

Chemicals:

Dulbecco's modified Eagle medium (DMEM) culture medium, trypsin, fetal bovine serum (FBS), and antibiotics/antimycotic solution were sourced from Himedia Laboratories, Mumbai. Plastic wares and other consumables used in the study were obtained from Tarsons, Kolkata. Galactosamine, MTT, and all specified chemicals and reagents were procured from Sigma Aldrich and SRL Chemicals.

Collection and preparation of the plants extracts:

Impatiens henslowiana was sourced from the Western Ghats of the Kerala region in South India. The plants underwent a meticulous process of examination, identification, and authentication. The entire plant was air-dried and subsequently transformed into a powder. About 500 g of the powdered sample from each medicinal plant was weighed and subjected to successive solvent extraction using hexane, chloroform, ethyl acetate, ethanol, and water in a Soxhlet apparatus. The extraction process was carried out for 3 days for each solvent. The resulting filtrate underwent evaporation to dryness at 40°C using a rotary evaporator. This



extraction procedure was repeated several times until a sufficient quantity of extract was obtained. The concentrated extract from each plant was then stored at 4°C until needed for use.

Phytochemical analysis:

Phytochemical screening was conducted using established procedures. The plant extracts were assessed for the presence of various phytoconstituents, including alkaloids, carbohydrates, tannins, saponins, terpenoids, flavonoids, and cardiac glycosides [12].

GC-MS analysis:

The GC-MS analysis of the plant extract was conducted using a Perkin-Elmer Clarus 680 system equipped with a fused silica column (Elite5MS capillary column, 30 m length × 250 μm diameter × 0.25 μm thickness). Helium served as the carrier gas at a constant flow rate of 1 mL/min. Compounds were detected in the GC-MS spectral range of 40 to 600 m/z, employing an ionization energy of 70 eV and a scan time of 0.2 s. The injector, maintained at 250°C, featured a constant injection volume of 1 μL. The column temperature initiated at 50 °C for 3 minutes, followed by an increase of 10 °C per minute until reaching 280 °C. The final temperature was held at 300 °C for 10 minutes. Identification of the compounds was based on a comparison of retention time, peak area, peak height, and mass spectral patterns with authentic compounds stored in the National Institute of Standards and Technology (NIST) library [13].

Anti-oxidant activity:

DPPH assay:

The DPPH assay is a simple, rapid, cost-effective, and widely used method for assessing antioxidant activity. Despite its involvement in hydrogen atom transfer, the fundamental chemical reaction in the DPPH assay is recognized as an electron transfer (ET) reaction. This distinction arises from the fact that the transfer of hydrogen from an antioxidant to DPPH is a relatively slow process, considered a marginal reaction path. In contrast, the electron transfer from a deprotonated antioxidant to DPPH is a faster and rate-determining step in this context [14].

In brief, a 0.135 mM DPPH solution was prepared in methanol. Various test samples at different concentrations (5, 10, 20, 40, 80, 160, and 320 μg/ml) and ascorbic acid were mixed with 2.5 ml of the DPPH solution. The reaction mixture was thoroughly vortexed and left at room temperature for 30 minutes.

Subsequently, the absorbance of the mixture was measured at 517 nm. The percentage of inhibition was calculated using the formula:

$$\% \text{ DPPH scavenging activity} = \frac{[(\text{OD of control} - \text{OD of test}) / (\text{OD of control})] \times 100}$$

ABTS assay:

The ABTS assay is suitable for evaluating the antioxidant capacity of both hydrophilic and lipophilic samples, given that ABTS is soluble in both water and organic solvents. In the presence of hydrogen-donating antioxidants, the blue/green ABTS undergoes reduction to colorless ABTS at 734 nm, and the extent of antioxidant activity is directly correlated with the reduction in absorbance [15]. The ABTS solution was created by combining 7 mM of ABTS and 2.45 mM of K₂S₂O₈ in water, followed by a dark incubation for 12–16 hours at room temperature. Prior to use, the ABTS solution was diluted with a water and ethanol solution to attain an absorbance of 0.7 ± 0.02 at 734 nm using a UV-visible spectrophotometer. In the assay, 2 mL of the ABTS solution was added to 100 μL of test samples at various concentrations. The mixtures were thoroughly blended, incubated at room temperature for 10 minutes, and the absorbance was promptly measured at 734 nm. The percentage inhibition of absorbance was calculated, and the data were plotted against the concentration of both the standard and the sample. IC₅₀ values, which represent the concentration at which 50% inhibition occurs, were determined using an appropriate formula. The radical scavenging activity was calculated using the equation:

$$\% \text{ ABTS scavenging activity} = \frac{[(\text{OD of control} - \text{OD of test}) / (\text{OD of control})] \times 100}$$

Preparation of Cell line and Cell viability assay:

Human liver hepatocellular carcinoma (HepG2) cell lines were procured from the National Centre for Cell Science (NCCS) in Pune, India. These cells were routinely cultured in DMEM supplemented with 10% FBS in a humidified atmosphere with 5% CO₂ at 37°C until reaching confluence. For cell dissociation, trypsin phosphate versene glucose solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS) was employed. The stock cultures were maintained in 25 cm² culture flasks, and all experiments were conducted in 96 microtiter plates. Cells were seeded at a density of 1×10⁵ cells/mL, and the culture medium was refreshed twice a week. Cell



viability was assessed before centrifugation. Subsequently, 50,000 cells/well were seeded in a 96-well plate and incubated for 24 hours at 37°C in a 5% CO₂ incubator. The IC₅₀ values for cytotoxicity tests were determined through nonlinear regression analysis (curve fit) based on the sigmoid dose–response curve (variable) and calculated using GraphPad Prism 6 [16].

Cytoprotective effects of plant extracts:

The cells were seeded at a density of 1.0×10^5 cells/mL in 96-well flat-bottomed plates and incubated at 37°C in a humidified incubator with 5% CO₂. After 24 hours, when a partial monolayer had formed, the supernatant was aspirated, and the monolayer was washed once. The final volume in all treated and control wells was maintained at 200 µl. Galactosamine (0.4%), silymarin (100 µg/ml), and various concentrations (25 µg/mL, 12.5 µg/mL) of plant extracts were added to the specified wells. Silymarin was used as a control. Microscopic examination was conducted after 60 minutes of Galactosamine intoxication. Cytotoxicity was assessed by determining the percentage viability of HepG2 cells using the MTT reduction assay. Absorbance was measured with an enzyme-linked immunosorbent assay reader at 540 and 630 nm [17]. Wells containing only medium served as a blank, while untreated wells were utilized as a control in the assay. Furthermore, for the protective potential of compounds on GSH levels and intracellular ROS generation against Galactosamine, cells were pre-exposed to the plant extract for 24 h, and then Galactosamine (0.1%) was added for an additional 24 h.

Determination of glutathione content

Glutathione (GSH) serves as an antioxidant tripeptide in hepatocytes, and its concentrations may decrease in the presence of reactive oxygen species (ROS). Briefly, cell lysates in cold KCl 1.15% were homogenized with Tris-HCl 25 mM pH 7.4 (2:1, v/v) and then incubated for 60 minutes at 37°C. Subsequently, the mixture was combined with TCA 10% (1:1, v/v) and subjected to centrifugation. A 100 µl aliquot of the supernatant was mixed with 180 µl of EDTA-phosphate buffer and 20 µl of 5,5'-dithio-bis 2-nitrobenzoic acid 5 mM (Ellman reagent). GSH levels were determined spectrophotometrically at 412 nm, inferred from a calibration curve, and adjusted for the protein level of cell lysates [18].

Measurement of malondialdehyde (MDA) content and SOD activity

In 6-well plates, a total of 1×10^6 cells were plated per well and allowed to adhere for 16 hours. Subsequently, the cells were exposed to the test samples for 30 minutes before being subjected to 0.4% Galactosamine for 6 hours. After the treatment, the cells underwent two washes with PBS, were suspended in 0.4 mL of PBS, and then sonicated for 20 seconds. The resulting homogenate was centrifuged at 6000 x g for 10 minutes, and the supernatant was collected for subsequent experiments. The determination of MDA content was conducted using the thiobarbituric acid reactive substances (TBARS) assay. Additionally, the superoxide dismutase (SOD) activity in the homogenate was evaluated using a commercial colorimetric SOD assay kit from Cayman Chemical Company [19].

4.Results & Discussion:

The powdered plant sample of *Impatiens henslowiana*, weighing 500 grams, underwent extraction using various solvent systems including hexane, ethyl acetate, chloroform, ethanol, and water. This method has demonstrated efficiency in extracting active compounds from plant species, particularly flavonoids, polyphenols, alkaloids, and steroids. The serial exhaustive extraction system, ranging from nonpolar to polar solvents, produced five extracts with yield values: Hexane (IBH) – 12.28g, Ethylacetate (IBEA) - 8.02g, Chloroform (IBC) – 1.69g, Ethanol (IBM) - 11.66g, and Water (IBW) – 11.02g, respectively. These yields varied depending on the extraction solvent used.

Phytochemical Analysis

The phytochemical screening of the three plant samples indicated the presence of alkaloids, tannins, flavonoids, cardiac glycosides and phenols. These phytochemicals, known for their potential medicinal activities on human health, tested positive in the analysis. The identification of flavonoids and terpenoids, previously reported for their anti-microbial, anti-mutagenic, anti-inflammatory, and anti-allergic properties in various studies, is noteworthy. This suggests that these plants may offer potential health benefits (Table -1).

GC-MS analysis:

The GC-MS analysis revealed a chromatogram of the plant extract, as depicted in Fig. 3. Table-2



Table – 1: Phytochemical analysis of *Impatiens henslowiana* extracts

Sample	Alkaloids	Flavonoids	Saponins	Tannins	Phenols	Cardiac glycosides	Steroids	Terpenoids	Quinones	Proteins
IBH	-	-	-	-	-	-	+	+	-	-
IBEA	+	+	-	+	+	+	-	-	+	-
IBC	+	+	-	-	+	+	+	+	+	+
IBE	+	+	-	+	+	+	+	+	+	+
IBW	+	+	-	+	+	+	+	+	+	-

provides details on the functional groups, molecular formula, molecular weight, and retention time of the respective compounds. The analysis identified 7 major compounds, including N-hexadecanoic acid; 9,12-tetradecadien-1-ol, acetate, (Z,E)-; 7-oxabicyclo[4.1.0]heptane, 1-methyl-4-(2-methyloxiranyl)-; oxo-4,6-diphenyl-1,2,3,4-tetrahydropyrimidin: 4-pentadecyne, 15-chloro; 6,7-dibromo-Z-11-dibromo-Z-1 tetradecene-1-ol acetate; 1-propene,2-nitro-3-(1-cyclooctenyl).

Table – 2: GCMS analysis of *Impatiens henslowiana* extract

S.NO	Name of the compound	RT	Molecular weight	Molecular formula
1.	N-HEXADECANOIC ACID	20.69	256	C16H32O2
2.	9,12-TETRADECADIEN-1-OL, ACETATE, (Z,E)-	21.83	252	C16H28O2
3.	7-OXABICYCLO[4.1.0]HEPTANE, 1-METHYL-4-(2-METHYLOXIRANYL)-	22.45	168	C10H16O2
4.	OXO-4,6-DIPHENYL-1,2,3,4-TETRAHYDROPYRIMIDIN	20.96	248	C14H16O4
5.	4-PENTADECYNE, 15-CHLORO	24.83	242	C15H27Cl
6.	6,7-DIBROMO-Z-11-TETRADECENE-1-OL ACETATE	24.90	410	C16H28O2Br2
7.	1-PROPENE, 2-NITRO-3-(1-CYCLOOCTENYL)	28.14	195	C11H17O2N

Antioxidant Activity Using DPPH Assay:

DPPH is a stable free radical characterized by absorption at 517 nm, and its absorption decreases significantly when exposed to proton radical scavengers. Due to its stability and simplicity, it has been widely used to evaluate antioxidative ability [20]. The assay was conducted in methanol, and the results are expressed as IC₅₀, representing the concentration of a sample scavenging 50% of the DPPH free radicals in a given experimental situation. The IC₅₀ value of the *Impatiens henslowiana* extracts IBH, IBEA, IBC, IBM, and IBW and the reference standard ascorbic acid were found to be >320 µg/ml, >320 µg/ml, >320 µg/ml, 220.4 µg/ml, >320 µg/ml, and 13.42 µg/ml, respectively (Fig-1). Furthermore, the results indicated that the DPPH free radical scavenging activity of ethanol extract was relatively more pronounced than that of Ethylacetate, water, hexane and chloroform extracts but weaker than that of L-ascorbic acid. The antioxidative property of these extracts may be attributed to the number of flavonoid and phenolic hydroxyl groups.

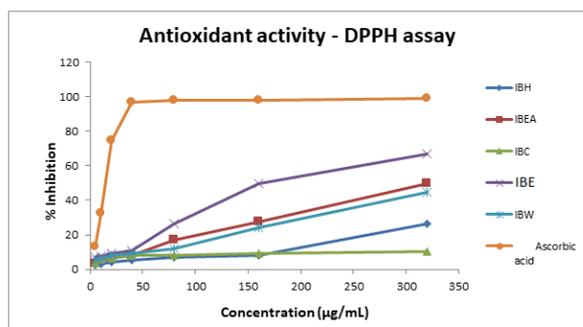


Fig – 1: DPPH radical scavenging assay of *Impatiens henslowiana* extracts

Antioxidant Activity Using ABTS Assay:

The outcomes illustrating the scavenging activity against free radicals of *Impatiens henslowiana* extracts are presented in Fig - 2. The order of descending ABTS scavenging activity for various extracts was as follows: (IBH, IBEA, IBC, IBM, and IBW) and the standard drug (Ascorbic acid) were found to be 92.86 µg/mL, 16.73 µg/mL, 82.39 µg/mL, 13.52 µg/mL, 24.40 µg/mL, and 9.51 µg/mL, respectively. The IC50 value of the ethanol extract was near to that of ascorbic acid and was lower than that of other extracts, indicating robust antioxidant activity in the ethanol extract [21].

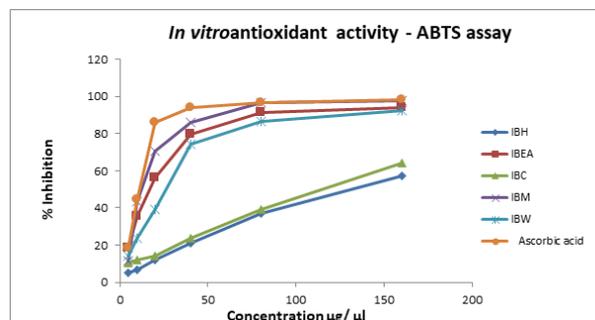


Fig – 2: ABTS radical scavenging assay of *Impatiens henslowiana* extracts

MTT Assay for HepG2 Cell Line:

The cytotoxicity of compounds was assessed through the MTT assay and examination of morphological changes. The study shows that the *Impatiens henslowiana* extracts did not affect the cell viability of HepG2 cells (Fig - 3) within the 25 µg/ml concentrations. In contrast, the plant extract exhibited cytotoxic effects at the higher concentrations of 100 µg/ml (data not shown). So the concentrations chosen for

the cytoprotective activity were 1.25 µg/ml to 25 µg/ml with 50% viability of the cells.

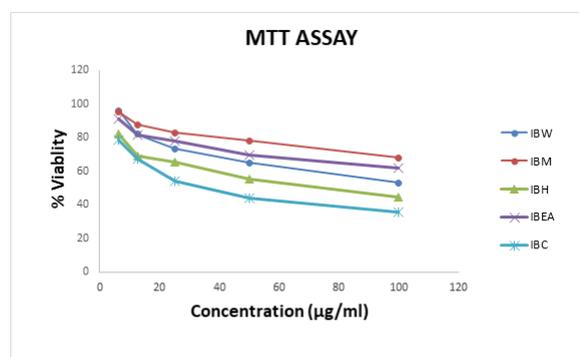


Fig – 3: *In vitro* cytotoxicity of *Impatiens henslowiana* extracts

Hepatoprotective activity of *Impatiens henslowiana* of Galactosamine-induced HepG2 cell line:

The HepG2 cell line is considered a suitable model for *in vitro* liver toxicity studies due to its retention of many specialized liver functions [22]. In this study, we initially examined the response of HepG2 cells to varying doses of Galactosamine using the MTT assay. The cell viability ratio of the control group was set at 100%. The results revealed that Galactosamine ranging from 0.1 to 1% induced cell death in a dose-dependent manner, with 0.4% Galactosamine mildly affecting cell viability (data not shown). Subsequent studies involved cell incubation with 0.4% Galactosamine for 6 hours to induce 40–50% cell death. There was no apparent cytotoxic or inhibitory effect on the growth of HepG2 cells with plant extracts based on the MTT assay.

As shown in Fig. 4, treatment with 0.4% Galactosamine alone resulted in cell death, indicating the sensitivity of HepG2 cells to Galactosamine. However, pre-treatment with the plant extract protected cells from Galactosamine-induced damage, restoring cell survival. At 6.25 µg/ml, the ethanol extract exhibited a 65.45% hepatoprotective effect, while the other extracts showed weaker efficacy even at higher concentrations. Additionally, the hepatoprotective potency of the ethanol extract at 6.25 µg/ml was comparable to that of the standard drug silymarin 88.62% (Fig. 5).

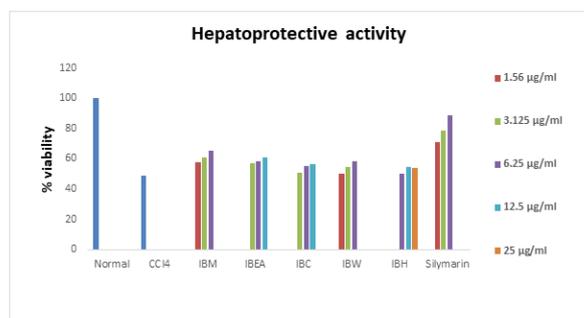


Fig – 4: *In vitro* Hepatoprotective activity of *Impatiens henslowiana* extracts

Effect of *Impatiens henslowiana* extracts on GSH level:

Glutathione plays a crucial role in counteracting oxidative species. Exogenous exposure to Galactosamine is known to elevate intracellular reactive oxygen species generation, leading to cellular oxidative damage in hepatic cells. This damage can be countered by hepatocyte antioxidant defense mechanisms. GSH, a crucial non-enzymatic antioxidant, plays a vital role in the cellular defense system against oxidative stress. Glutathione peroxidase catalyzes the oxidation of GSH to GSSG in the presence of Galactosamine, and glutathione reductase recycles oxidized GSH back to reduced GSH [23]. Following a 24-hour treatment period, both the plant extract and silymarin exhibited a significant increase in GSH content compared to the normal control. In the negative control, Galactosamine reduced GSH content by 52.28%. The presence of the ethanol extract established a preventive effect against oxidative stress by enhancing GSH content by 64.38% in Galactosamine-injured HepG2 cells. Notably, silymarin at 6.25 µg/ml significantly increased the GSH amount by up to 82.19% compared to the Galactosamine induced control group (Fig. 6). Therefore, the pre-exposure of HepG2 cells to the plant extract of *Impatiens henslowiana* moderately elevates glutathione levels, enhances antioxidant status, and protects cells against Galactosamine-induced damage.

Effect of *Impatiens henslowiana* extracts on MDA level:

The toxicity induced by Galactosamine in the HepG2 cell line can be attributed to either a direct solvent effect or the generation of free radicals, leading to the initiation of lipid peroxidation. Lipid peroxidation, a

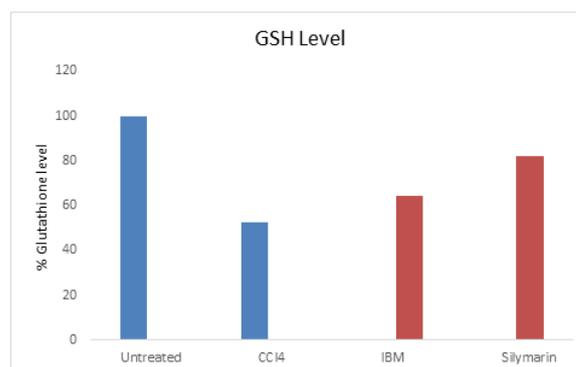


Fig – 6: Hepatoprotective activity of *Impatiens henslowiana* extracts on GSH level induced by Galactosamine in HepG2 cell line

process involving the oxidative alteration of polyunsaturated fatty acids in cell membranes, gives rise

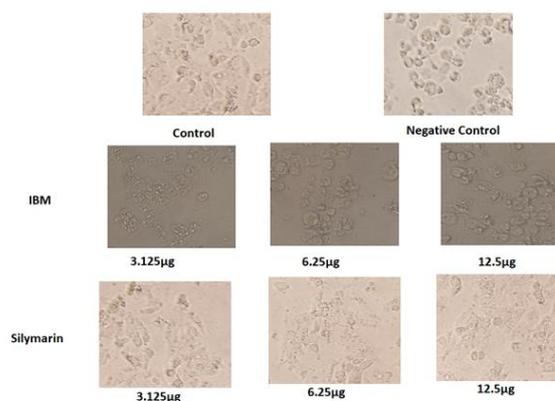


Fig – 5: HepG2 cell line morphology on Hepatoprotective activity of *Impatiens henslowiana* extracts

to various free radicals. The lipid radical is rapidly quenched by molecular oxygen, forming a peroxy-fatty acid radical that can generate end products such as MDA and unsaturated aldehydes [24]. To understand the consequences of Galactosamine-induced oxidative damage to cellular macromolecules and explore the protective effects of *Impatiens henslowiana*, the formation of MDA was assessed. As depicted in Fig. 7, the level of MDA significantly increased to 198.25% in the negative control upon treatment with Galactosamine. In contrast, pre-treatments with ethanol extracts and silymarin inhibited Galactosamine-induced lipid peroxidation in HepG2 cells to 169.34% and 123.48%, respectively. These findings suggest that *Impatiens henslowiana* has the potential to suppress intracellular MDA formation induced by Galactosamine, with the ethanol extract exhibiting a moderate cytoprotective effect.

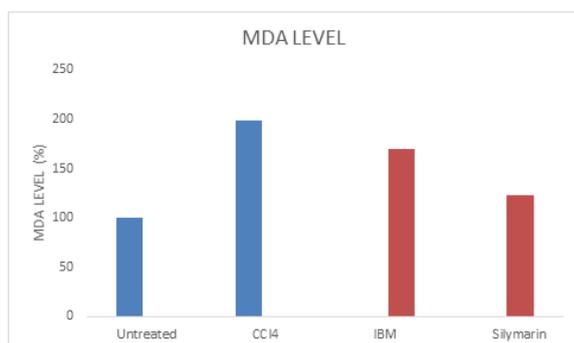


Fig – 7: Hepatoprotective activity of *Impatiens henslowiana* extracts on MDA level induced by Galactosamine in HepG2 cell line

Effect of *Impatiens henslowiana* extracts on SOD activity

The efficacy of *Impatiens henslowiana* against Galactosamine-induced hepatotoxicity in HepG2 cells was assessed by investigating their impact on antioxidative enzymes. The study focused on SOD, which is a crucial component of the cellular defense against oxidative stress [25]. SOD, in particular, plays a pivotal role in catalyzing the dismutation of superoxide radicals, a vital process in mitigating oxidative stress. The hepatoprotective effects of plant extracts may involve the modulation of these endogenous antioxidant enzymes. The results, as depicted in Fig. 8, reveal that exposure of HepG2 cells to Galactosamine significantly decreased SOD activities to 34.26% in the negative control group. However, pre-treatment with the ethanol extract and silymarin significantly alleviated this decrease in SOD activities to 51.62% and 82.64%, respectively, compared to the untreated group.

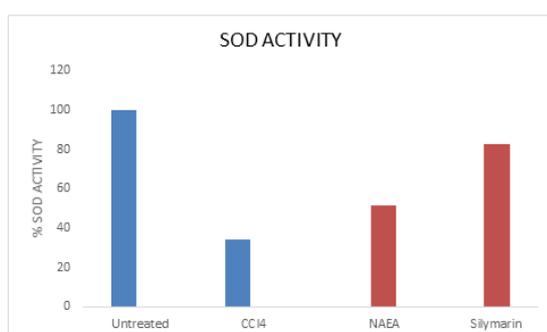


Fig – 8: Hepatoprotective activity of *Impatiens henslowiana* extracts on SOD activity induced by Galactosamine in HepG2 cell line

5. Conclusion:

In conclusion, the study highlighted the hepatoprotective effects of *Impatiens henslowiana* extracts, particularly the significant impact observed in ethanol extract-treated HepG2 cells subjected to Galactosamine-induced injury. This protective effect appears to be associated with the antioxidant activity of the extracts. The findings underscore the importance of further investigations to assess the liver protective properties of *Impatiens henslowiana*, potentially extending to animal models and clinical trials.

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CONFLICTS OF INTERESTS

The authors declared there are no conflicts of interest.

References

- Xinsheng Gu and Jose E. Manautou, Molecular mechanisms underlying chemical liver injury. *Exp. Rev. Mol. Med.* **14**, 25 (2012); <https://doi.org/10.1017/s1462399411002110>
- Marianthi G. Lerapetritou, Panos G. Georgopoulos, Charles M. Roth, Loannis P. Androulakis. *Clin. Transl. Sci.* **2**, 228 (2009); <https://doi.org/10.1111/j.1752-8062.2009.00092.x>
- Marc G Sturgill, George H Lambert, *Clin. Chem.* **43**, 1512 (1997); <https://doi.org/10.1093/clinchem/43.8.1512>
- Ruidong Li, Wenchang Yang, Yuping Yin, Xianxiong Ma, Peng Zhang and Kaixiong Tao. *Front. Pharmacol.* **12**, 1 (2021); <https://doi.org/10.3389/fphar.2021.651444>
- Wei, Y.; Wang, H.; Zhang, Y.; Gu, J.; Zhang, X.; Gong, X.; Hao, Z. *Antioxid.* **11**, 2234 (2022); <https://doi.org/10.3390/antiox11112234>
- S A Sheweita, M A El-Gabar, M Bastawy *Toxicol* **169**, **83** (2001); [https://doi.org/10.1016/s0300-483x\(01\)00473-5](https://doi.org/10.1016/s0300-483x(01)00473-5)



7. B. Dheeba, P. Sampathkumar, S. Vijay, K. Kannan, M. Kannan, *Asian J. Chem* **26**, 230 (2014); <https://doi.org/10.14233/ajchem.2014.15755>
8. Jie Cai, Jie Peng, Juan Feng, Ruocheng Li, Peng Ren, Xinwei Zang, Zezong Wu, Yi Lu, Lin Luo, Zhenzhen Hu, Jiaying Wang, Xiaomeng Dai, Peng Zhao, Juan Wang, Mi Yan, Jianxin Liu, Renren Deng, Diming Wang, *Nat. Comm.* **14**, 3643 (2023); <https://doi.org/10.1038/s41467-023-39423-3>
9. Jyosna R.N. Dessai and M.K. Janarthanam, *Rheedea* **21**(1):23-80; <https://www.researchgate.net/publication/258506812>
10. Paulraj Selva Singh Richard a, Subbiah Karuppusamy b, Vel lingiri Ravichandran, *J. Asia-Pacific Biodiver.* **15**, 138 (2022); <https://doi.org/10.1016/j.japb.2021.12.001>
11. Prashasti Tripathi, Anupam Tripathi, Vinita Bisht and Shalini Purwar, *Pharm. Innov. J.* **9**, 293, (2020); <https://www.thepharmajournal.com/archives/2020/vol9issue8/PartD/9-8-40-568.pdf>
12. Rajendran K Selvakesavan, Gregory Franklin *Nanotechnol Sci Appl.* **14**, 179 (2021); <https://doi.org/10.2147%2FNSA.S333467>
13. Sruthy Elsa Madhu a, H. Sreeja b, Joyce Sudendara Priya, *Mat. Tod. Proceed.* **25**, 343, (2020); <https://doi.org/10.1016/j.matpr.2020.03.157>
14. Erum Iqbal a, Kamariah Abu Salim b, Linda B.L. Lim, *J King Saud. Univ.* **27**, 224 (2015); <https://doi.org/10.1016/j.jksus.2015.02.003>
15. Ibrahim Chikowe, King David Bwaila, Samuel Chima Ugbaja, Amr S Abouzied, *Sci. Rep.* **14**, 1876 (2024); <https://doi.org/10.1038/s41598-023-47737-x>
16. Endris Muhie Hussen and Sisay Awoke Endalew, *BMC Comp. Med. Ther.* **23**, 146 (2023); <https://doi.org/10.1186%2Fs12906-023-03923-y>
17. Mavie Rose Kongolo Kalemba, Rhulani Makhuvele, Patrick Berka Njobeh, *Heliy.* **10**, e24435 (2024); <https://doi.org/10.1016/j.heliyon.2024.e24435>
18. Sasipawan Machana, Natthida Weerapreeyakul, Sahapat Barusrux, Apiyada Nonpunya, Bungorn Sripanidkulchai, Thaweesak Thitimetharoch, *Chin. Med.* **6**, 39 (2011); <http://www.cmjournal.org/content/6/1/39>
19. Ezzat MI, Okba MM, Ahmed SH, El-Banna HA, Prince A, Mohamed SO, *PLoS.* **15**, e0226185 (2020); <https://doi.org/10.1371/journal.pone.0226185>
20. Vaishali N. Shah, Mamta B. Shah & Parloop A. Bhatt, *Pharm. Bio.*, **49**, 408 (2011); <http://informahealthcare.com/doi/abs/10.3109/13880209.2010.521162>
21. Liliana Torres González, Noemí Waksman Minsky, Linda Elsa Muñoz Espinosa, Ricardo Salazar Aranda, Jonathan Pérez Meseguer and Paula Cordero Pérez, *BMC Comp. Alt. Med.* **17**, 39 (2017); <https://doi.org/10.1186%2Fs12906-016-1506-1>
22. Siddartha Baliyan, Riya Mukherjee, Anjali Priyadarshini, Arpana Vibhuti, Archana Gupta, Ramendra Pati Pandey, Chung-Ming Chang, *Molecules* **27**, 1326 (2022); <https://doi.org/10.3390%2Fmolecules27041326>
23. R Re, N Pellegrini, A Proteggente, A Pannala, M Yang, C Rice-Evans, *Free Rad. Biol. Med.* **26**, 1231 (1999); [https://doi.org/10.1016/s0891-5849\(98\)00315-3](https://doi.org/10.1016/s0891-5849(98)00315-3)
24. R Krithika, RJ Verma, PS Shrivastav, *Human Exp. Toxicol.* **32**, 530 (2013); <https://doi.org/10.1177/0960327112459530>
25. Jayaraman Rajangam, A.J.M.Christina, *J. Appl. Pharm. Sci.*, **3**, 069 (2013); <https://doi.org/10.7324/JAPS.2013.30212>
26. Rania Abdel Rahman Elgawish, a Haidy G. Abdel Rahman, Heba M.A. Abdelrazek, *Toxicol Rep.* **2**, 1149 (2015); <https://doi.org/10.1016%2Fj.toxrep.2015.08.001>
27. Min-Ji Bak, Mira Jun, Woo-Sik Jeong *Int. J. Mol. Sci.*, **13**, 2314 (2012); <https://doi.org/10.3390/ijms13022314>