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Mercury-Induced Structural Alterations and RuBisCO–Metal Interaction in Grewia asiatica L.: A Microscopic and Molecular Docking Study

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Abstract

Mercury (Hg^{2+}) is a highly toxic metal of growing environmental concern due to its persistence, bioaccumulation, and widespread release from industrial and agricultural sources. The use of green plants for *in situ* remediation offers a sustainable and cost-effective alternative to conventional mercury management strategies. This study investigates the mercury tolerance potential of *Grewia asiatica* L., a medicinal shrub, through integrated anatomical and bioinformatics approaches. Seedlings were exposed to 1000 ppm mercuric chloride ($HgCl_2$) under controlled conditions, and anatomical responses in leaf and root tissues were assessed using Field Emission Scanning Electron Microscopy (FESEM). Treated samples showed marked structural abnormalities, including guard cell swelling, stomatal distortion, and disorganization of root parenchyma, indicative of mercury-induced cellular stress. To complement the anatomical analysis, *in silico* studies focused on the ribulose bisphosphate carboxylase (RuBisCO) a large subunit protein (UniProt ID: A0A223AIW3). The 182-residue protein exhibited stable physicochemical characteristics and was modelled using AlphaFold. Structure validation yielded strong metrics (PROCHECK: 93.3%, Verify3D: 83.52%). Molecular docking via CB-Dock2 revealed a high-affinity interaction between RuBisCO and Hg^{2+} ions, suggesting a possible role in metal binding or detoxification. These findings, derived from both anatomical observations and computational modelling, provide novel insights into the mercury stress response of *Grewia asiatica* and support its potential use in phytoremediation strategies for mercury-contaminated environments.

Keywords: *Grewia asiatica*, Mercury Stress, FESEM, RuBisCO, Molecular Docking, Environmental Detoxification

Introduction

Metals are essential components of living cells. Elements like sodium and potassium regulate ion gradients across membranes, while copper, iron, and manganese act as cofactors for metalloenzymes involved in photosynthesis and electron transport. However, excessive concentrations of metals can disrupt cellular processes, affecting growth, morphology, and metabolism. Non-essential metals such as arsenic, cadmium, chromium, lead, mercury, and uranium often referred to as heavy metals due to their atomic number above 20 and density greater than 5 g/cm³ are particularly toxic. Although heavy metals occur naturally, anthropogenic activities such as industrial discharge, mining, excessive agrochemical use, and improper waste disposal have drastically elevated their environmental levels. Agricultural soils are particularly at risk due to practices like the application of sewage sludge, industrial by-products, and irrigation with wastewater [1-4]. These pollutants persist in ecosystems, posing risks to plants, animals, and humans through bioaccumulation and trophic transfer [5]. While several plant species exhibit mechanisms for metal tolerance such as

accumulation, sequestration, or biochemical transformation these processes are highly species and context dependent. Mercury, a highly toxic metal, is ubiquitously present in the environment, particularly in soluble forms like mercuric chloride ($HgCl_2$) [6,7]. Its toxicity is linked to its strong affinity for thiol (-SH) groups in proteins, disrupting essential cellular functions such as enzymatic activity and gene expression. In this study, *Grewia asiatica* L. a medicinal berry plant native to the Indian subcontinent was selected to evaluate its potential in environmentally friendly mercury management. The plant was taxonomically identified and authenticated by the Herbarium Centre, (P.G.) Department of Botany, Ramananda College, under Bankura University.

Scientific Classification

Domain:	Eukaryota
Kingdom:	Plantae
Phylum:	Spermatophyta
Sub-phylum:	Angiosperma
Class:	Dicotyledonae
Order:	Malvales
Family:	Tiliaceae
Genus:	*Grewia*
Species:	*Grewia asiatica*

Seedlings were grown in $HgCl_2$-treated soil, and leaf and stem sections were examined using Field Emission Scanning Electron Microscopy (FESEM). Microstructural changes observed under mercury stress suggest a possible tolerance and accumulation response. Additionally, qualitative phytochemical screening of aqueous extracts from leaves, fruits, and flowers revealed the presence of secondary metabolites, which may contribute to stress mitigation. To complement anatomical and phytochemical assessments, a molecular-level investigation was incorporated to understand potential protein-level responses of *Grewia asiatica* to mercury stress. Among plant proteins, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) plays a pivotal role in the Calvin cycle and carbon assimilation. Beyond its photosynthetic function, recent studies suggest that RuBisCO and other metabolic enzymes may undergo structural adaptations under abiotic stress, including heavy metal exposure [8,9]. Its abundance and reactive surface residues make it a candidate for potential interaction with toxic ions such as Hg^{2+} [9-12]. Advances in bioinformatics and molecular modelling have enabled in silico exploration of protein-metal interactions, offering insights into how protein conformation and binding properties may contribute to stress tolerance mechanisms [13,14]. Computational tools such as SOPMA for secondary structure prediction, AlphaFold for 3D modeling, and molecular docking platforms like CB-Dock2 have been widely applied to investigate protein functionality and ligand-binding behavior under environmental stress conditions [15,16].

In this context, the present study integrates anatomical, phytochemical, and computational approaches to evaluate the adaptive response of *Grewia asiatica* under mercuric chloride exposure. Emphasis is placed on understanding both structural alterations and the possible involvement of RuBisCO in metal interaction, thereby contributing to a holistic understanding of the plant's stress physiology and its potential for environmental detoxification applications.

Materials and Methods

Exposure of *Grewia asiatica* to Mercury Stress

To evaluate the heavy metal tolerance and accumulation characteristics of *Grewia asiatica* L., a 1000 ppm mercuric chloride ($HgCl_2$) working solution was prepared by dissolving analytical-grade $HgCl_2$ in distilled water to make 1000 mL of solution. The selection of 1000 ppm was based on the work of Baker and Brooks (1989), who reported threshold concentrations for hyper accumulation of various metals, including mercury, in plant tissues [17]. Similar concentrations have also been used in mercury toxicity studies involving anatomical and biochemical analysis in plants [9,18,19]. Two young, healthy *Grewia asiatica* L. plants of similar size were selected for the experiment. One plant was designated as the control (untreated), while the second was subjected to mercury stress by irrigating the soil with the 1000 ppm $HgCl_2$ solution. Both plants were maintained under identical environmental conditions and incubated for five days. After the treatment period, no significant visual toxicity symptoms such as leaf chlorosis, necrosis, or wilting were observed in either the treated or control plants, indicating tolerance or delayed toxicity onset at the applied concentration and duration. Following incubation, both plants were carefully uprooted. The roots were thoroughly rinsed with distilled water to remove adhering soil particles. Transverse sections of leaves and roots from both control and treated plants were prepared for microscopic analysis. Tissue dehydration was carried out using a graded ethanol series, following the protocol described by Dykstra et al. (2003), prior to Field Emission Scanning Electron Microscopy (FESEM) imaging [20].

Bioinformatics and Molecular Docking Analysis

Retrieval and Domain Identification

The amino acid sequence of the RuBisCO large subunit from *Grewia asiatica* (UniProt Accession: A0A223AIW3) was retrieved from the UniProt database. Conserved domain identification was performed using the Conserved Domain Database (CDD) provided by NCBI [21].

Primary Sequence Characterization

Physicochemical properties of the RuBisCO protein including molecular weight, theoretical isoelectric point (pI), aliphatic

index, instability index, and GRAVY were calculated using the ExPASy ProtParam tool [22].

Secondary Structure Prediction

To gain insight into the folding nature and structural motifs of the RuBisCO large subunit protein, secondary structural elements such as α-helices, β-sheets, turns, and random coils were predicted using the Self-Optimized Prediction Method with Alignment (SOPMA) tool [15]. This analysis provides a detailed understanding of the protein's conformational features, which are critical for its stability and potential interaction with heavy metal ions like Hg^{2+}.

Tertiary Structure Prediction and Quality Assessment

The 3D structure of the RuBisCO protein was predicted using AlphaFolda deep learning-based tool for high-accuracy protein modelling and refined with GalaxyWeb server [14,23]. Model validation was conducted using:

- PROCHECK for Ramachandran plot analysis [24].
- ERRAT to evaluate non-bonded interactions [25].
- Verify3D to assess compatibility between 3D models and their amino acid sequences [26].

Ligand Retrieval, Preparation and Characterization

The 2D structure of the mercury ion (Hg^{2+}) was obtained from the PubChem database (CID: 24085) in SDF format. File format conversion to PDB and energy minimization were performed using Open Babel and PyRx, respectively [27,28].

Molecular Docking

Blind docking of Hg^{2+} with the RuBisCO structure was carried out using CB-Dock2, which integrates cavity detection and docking with AutoDock Vina [16]. The resulting complex was visualized and analysed for interacting residues using Biovia Discovery Studio 2024.

Result

Structural Effects of Mercury Stress on *Grewia asiatica* L. (FESEM Analysis)

After proper dehydration, treated and untreated root and leaf sections of *Grewia asiatica* L. were examined using Field Emission Scanning Electron Microscopy (FESEM) at the University Science Instrumentation Centre (USIC), The University of Burdwan.

FESEM study of Leaf Sections

Control leaf sections (Figure 1) displayed normal stomatal structures with no visible damage. In contrast, leaf sections treated with 1000 ppm $HgCl_2$ (Figure 2) showed damaged stomata and swelling of the guard cells. Clear stomatal structures and normal venation were evident in control samples (Figure 3), while treated samples (Figure 4) showed shrinkage and deformities around ruptured stomata. These mercury-induced structural changes suggest impairment of stomatal function and altered leaf surface integrity.

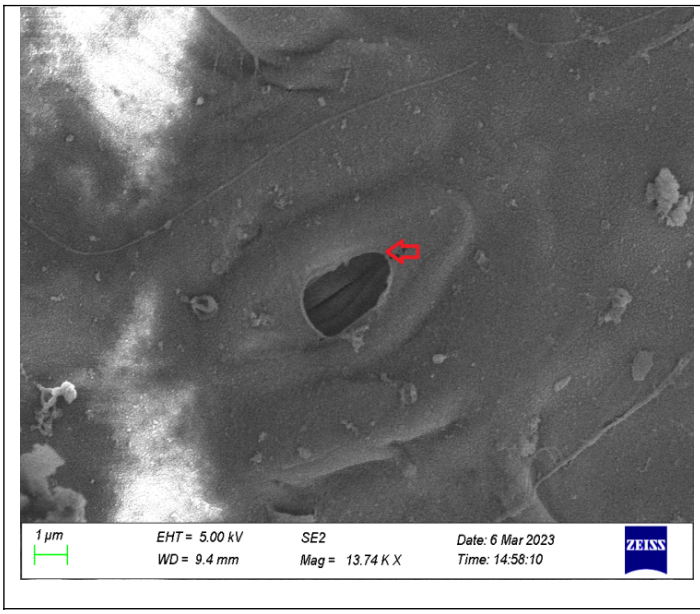

Figure 1: Control Leaf Section (Leaf Stomata is Clear, no Deformation Occurred)

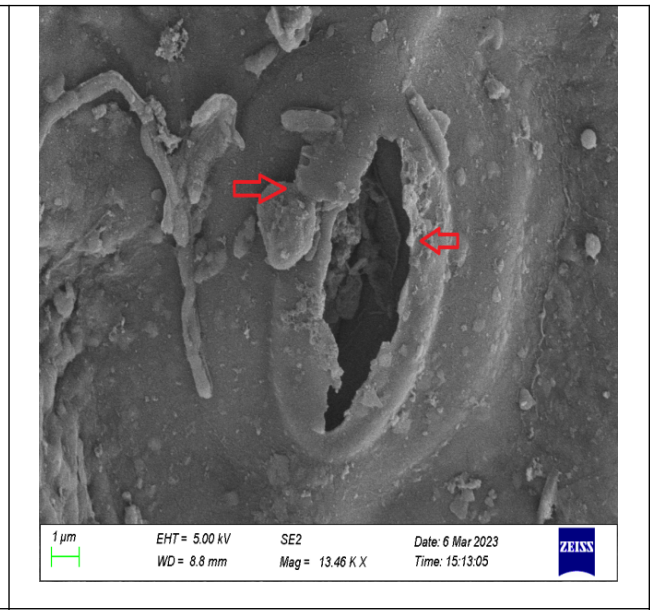

Figure 2: 1000ppm $HgCl_2$ Treated Leaf Section (Damaged Stomata and Swelling of the Guard Cells)

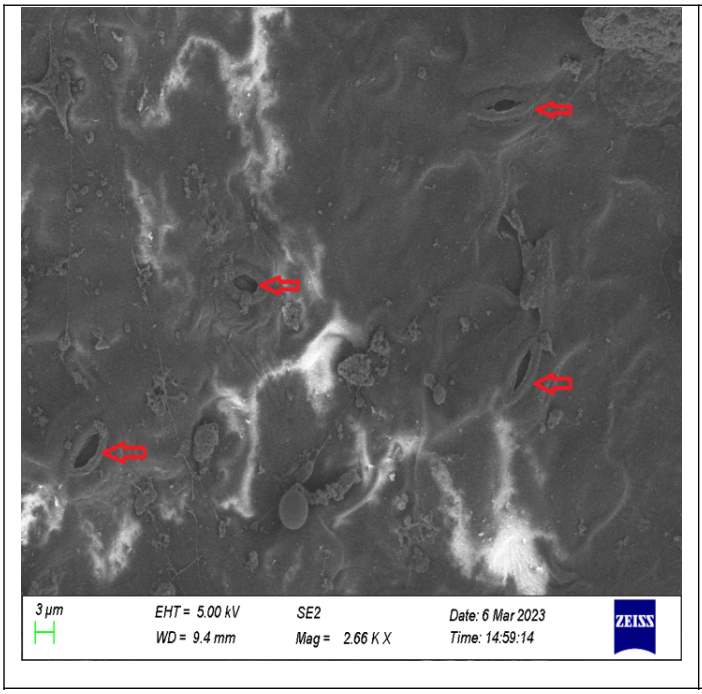

Figure 3: Clear Stomatal Structures were observed and Surrounding Area Appears Normal

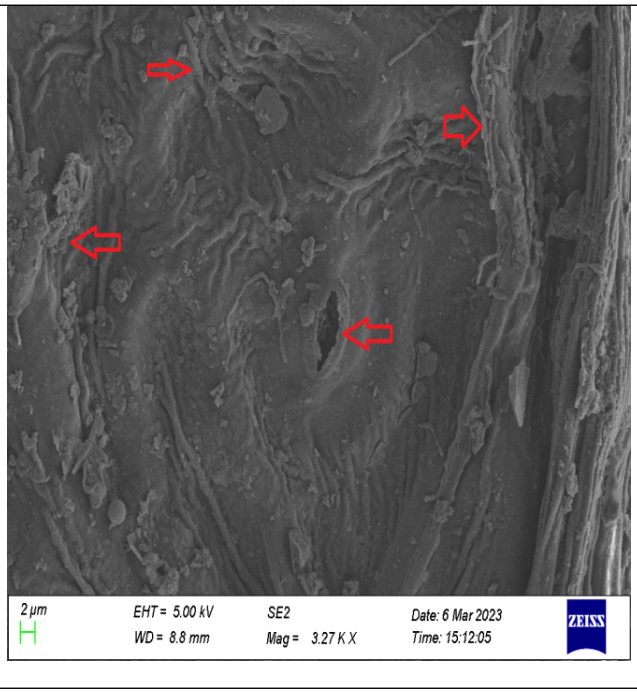

Figure 4: Deformities on the Surface Surrounding the Ruptured Stomata

FESEM Study of Root Sections

Control root sections exhibited intact, hexagonal parenchymal cells (Figure 5 and Figure 7). However, treated root sections (Figure 6 and Figure 8) showed ruptured and deformed parenchymal structures under 1000ppm $HgCl_2$ exposure. These changes imply disruption in root architecture, which may affect water and nutrient transport functions of the plant.

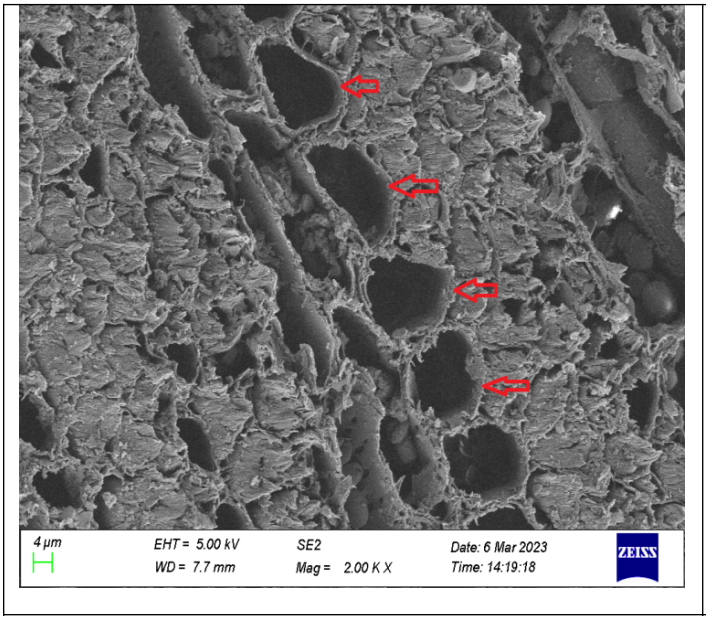

Figure 5: Clear Hexagonal Parenchymal Cells of Control Root Section

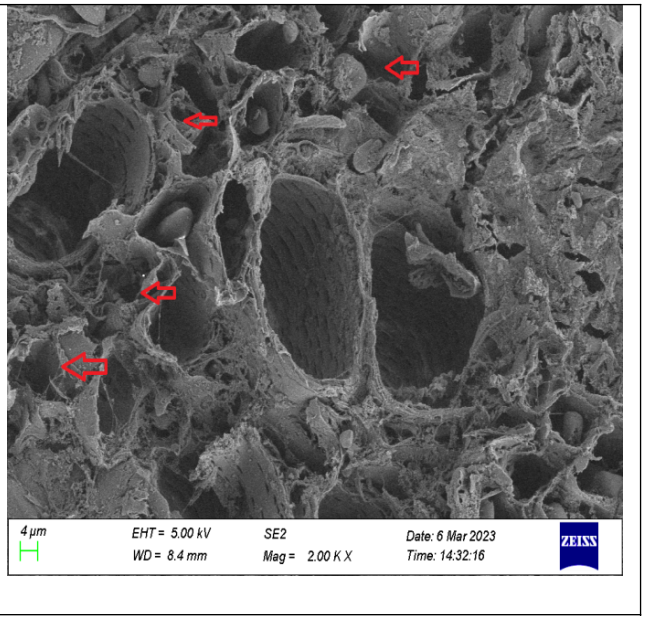

Figure 6: Damaged Parenchymal Cells of the Root Under 1000ppm $HgCl_2$stress

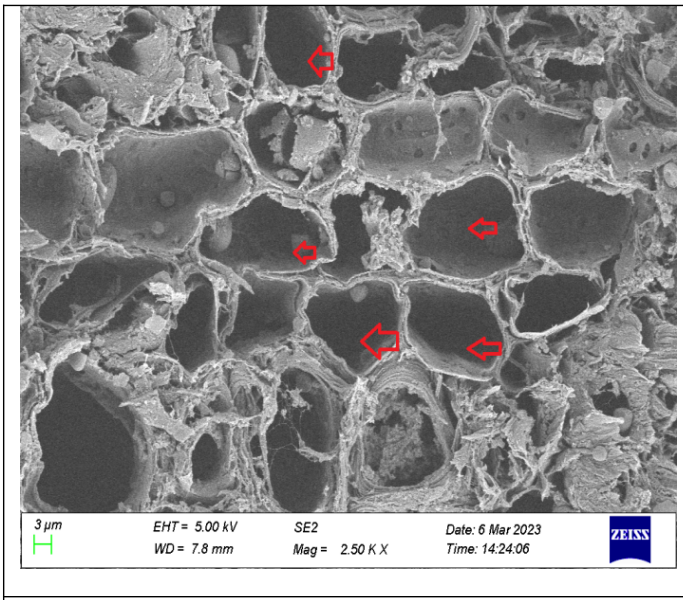

Figure 7: Normal Parenchymal Structures of Control Root Section

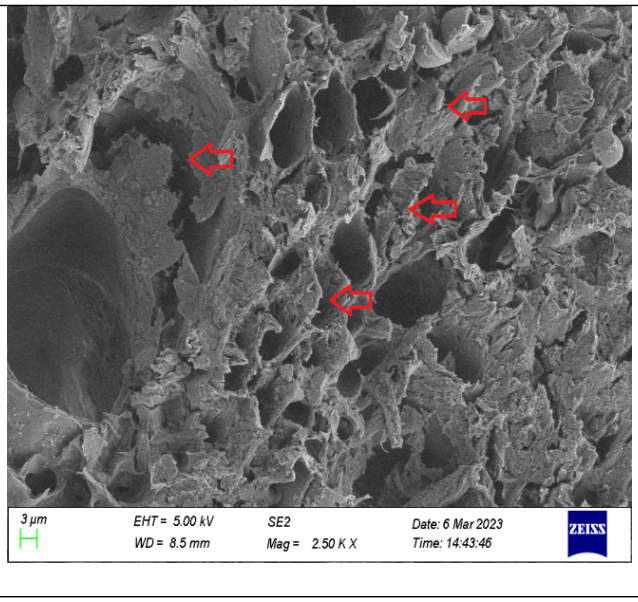

Figure 8: Deformed Parenchymal Cells of the Root Under 1000ppm $HgCl_2$ stress

In Silico Analysis of RuBisCO Protein

To investigate the molecular response of *Grewia asiatica* to mercury exposure, the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) large subunit protein (UniProt ID: A0A223AIW3) was analysed using bioinformatics tools.

Domain Identification

The amino acid sequence of RuBisCO was retrieved from the UniProt database. Conserved domain analysis using NCBI's Conserved Domain Database (CDD) confirmed the presence of functional motifs involved in carbon fixation, consistent with RuBisCO's enzymatic role.

Primary Sequence Characterization

Physicochemical characterization using ProtParam indicated a molecular weight of 20,355.14 KDa, an isoelectric point (pI) of 5.78, and an instability index of 29.31, suggesting the protein is stable under physiological conditions. The GRAVY value of -0.290 reflects a hydrophilic nature, favouring solubility in the cellular environment (Table 1).

Name	Accession no	No of amino acids	Molecular weight	Theoretical pI	-R	+R	Instability index:	Aliphatic	GRAVY index
Ribulose bisphosphate carboxylase large chain	A0A223AIW3	182	20355.14	5.78	22	20	29.31	76.10	-0.290

Table 1: Physicochemical Properties of the RuBisCO Large Subunit Protein from *Grewia asiatica*

Secondary and Tertiary Structure Prediction

Secondary structure analysis via SOPMA revealed that the protein comprises 26.37% α-helices, 14.84% β-strands, and 58.79% random coils, suggesting a well-organized and functional fold (Table 2). The tertiary structure was modelled using Alpha Fold and validated using multiple structure assessment tools. The model exhibited an ERRAT score of 93.29%, PROCHECK analysis indicated 93.3% of residues in favoured regions, and the Verify3D score was 83.52%, confirming high structural quality (Table 3, Figure 9).

Name	Alpha helix	Extended strand	Random Coil
Ribulose bisphosphate carboxylase large chain	48 (26.37%)	27 (14.84%)	107 (58.79%)

Table 2: Predicted Secondary Structural Elements of the RuBisCO Large Subunit Protein from *Grewia asiatica*

Name	Accession number	PROCHECK score (%)	Verified 3D score (%)	ERRAT score (%)
Ribulose bisphosphate carboxylase large chain	A0A223AIW3	93.3	83.52	93.29

Table 3: Model Validation Parameters of the RuBisCO Large Subunit Protein from *Grewia asiatica*

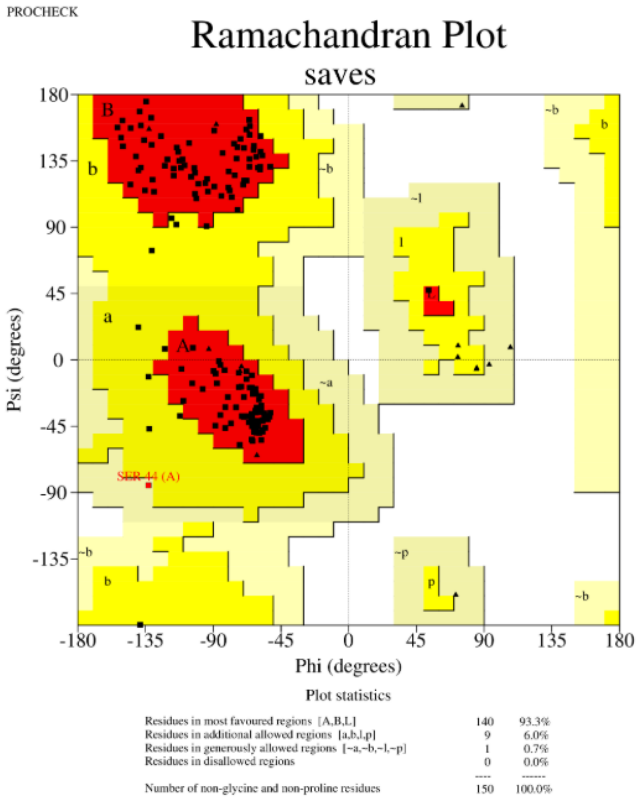

Figure 9: PROCHECK Analysis of Modelled Proteins Through Interpreting the Stereochemical Quality of Protein Structure

Molecular Docking with Mercury Ion (Hg^{2+})

Molecular docking between Hg^{2+} and the RuBisCO model was carried out using CB-Dock2, integrates cavity detection with AutoDock Vina to predict involved binding energy between ligand–protein and suitable poses of docked structure. Binding cavity predictions and docking scores guided interaction analysis, which was further visualized using Biovia Discovery Studio 2024. It has been found that mainly electrostatic bond was formed between RuBisCo and $HgCl_2$.

Discussion

Heavy metal contamination severely impacts plant systems by inducing oxidative stress, disrupting cellular ultrastructure, and impairing key metabolic and physiological functions. Metals such as mercury (Hg), cadmium (Cd), lead (Pb), and arsenic (As) are known to interfere with nutrient uptake, chlorophyll biosynthesis, and cellular integrity by generating reactive oxygen species (ROS) and binding to functional groups in enzymes and proteins [5]. In recent years, there has been growing interest in identifying plant species capable of tolerating or mitigating heavy metal toxicity through anatomical, biochemical, and molecular mechanisms [9,29]. In the present study, *Grewia asiatica* L. was exposed to 1000 ppm mercuric chloride ($HgCl_2$) to assess its anatomical and molecular responses to mercury-induced stress. The selected concentration was based on the hyperaccumulation threshold proposed by Baker and Brooks (1989), who identified 1000 ppm as a benchmark for evaluating metal tolerance in plant systems [17]. Similar concentrations have been used in studies exploring anatomical and biochemical alterations in response to mercury toxicity [18,19]. FESEM analysis of treated leaf tissues revealed significant stomatal deformation and guard cell swelling, which are indicative of impaired gas exchange and transpiration. Similar structural damage has been reported in *Cicer arietinum* and *Vigna radiata* under cadmium and mercury exposure, suggesting that stomatal architecture is among the first targets of heavy metal stress [30,31]. In root tissues, distortion of parenchymal cells and disrupted cellular outlines were evident, pointing toward compromised membrane integrity and loss of turgidity. These changes reflect a general pattern seen in other studies involving plants such as *Brassica juncea* and *Phaseolus vulgaris* exposed to Pb and Cr, where damage to vascular bundles and cortical cells impaired water and nutrient conduction [32,33].

Beyond anatomical responses, our study explored molecular mechanisms using *in silico* approaches. The RuBisCO large subunit protein from *Grewia asiatica* (UniProt ID: A0A223AIW3) was characterized to assess its structural behaviour and potential involvement in mercury interaction. Primary sequence analysis indicated the protein to be stable (instability index: 29.31), hydrophilic (GRAVY: –0.290), and thermally stable (aliphatic index: 76.10), all of which are beneficial features for proteins functioning under abiotic stress conditions. These observations are consistent with earlier studies where stress-responsive enzymes like superoxide dismutase (SOD) and glutathione reductase (GR) displayed enhanced solubility and thermal stability in metal-tolerant species [34]. Secondary structure analysis indicated that 26.37% of residues form α-helices and 58.79% comprise random coils, potentially reflecting conformational flexibility that allows functional adaptation under abiotic stress [35,36]. Proteins with high coil content often exhibit dynamic

regions that may accommodate interactions with metal ions or facilitate structural rearrangements under stress conditions [37]. The tertiary structure, predicted via AlphaFold and validated using multiple structure assessment tools, exhibited high structural reliability with 93.3% of residues in favored regions (PROCHECK), an ERRAT score of 93.29, and a Verify3D score of 83.52% (Table 3, Figure 9). These scores collectively confirm the accuracy of the predicted 3D conformation, which is essential for reliable molecular docking analysis [24-26]. High-quality structure prediction is particularly important when investigating protein–metal interactions, as stress-induced conformational changes may significantly influence metal binding sites and interaction stability [14,16]. Docking studies revealed a docking energy (ΔG) of –9.2 kJ/mol between Hg^{2+} and the RuBisCO protein and primarily interacted through electrostatic and metal acceptor bond within identified binding cavities (Table 4, Figures 10a and 10b). Key interacting residues Asp17, Tyr11, and Arg65 formed stabilizing contacts with the mercury ion, suggesting their role in metal coordination and binding stability. Similar protein-metal interactions have been reported in earlier studies, and have also been observed in other plant systems. In *Oryza sativa* (rice), arsenic and cadmium were shown to bind to photosynthetic and ribosomal proteins, leading to enzyme inhibition but also suggesting sequestration potential [38]. Similarly, in *Arabidopsis thaliana*, metalloproteins were identified as key components in detoxification pathways, aiding in metal compartmentalization and reducing cytotoxicity [39]. Our findings indicate that RuBisCO in *Grewia asiatica* may not only fulfil its classical role in carbon fixation but also participate in detoxification through transient binding or sequestration of toxic metal ions. This aligns with a broader understanding that proteins with accessible binding cavities, surface-exposed residues, or reactive thiol groups can act as metal buffers under stress conditions [8]. Numerous studies support this dual-role hypothesis. For example, metallothioneins and phytochelatins have long been recognized for their metal-binding capabilities, and recent proteomic analyses have highlighted non-canonical roles for housekeeping enzymes in abiotic stress responses, including ROS detoxification and metal homeostasis [40-42]. Our bioinformatics findings are, therefore, consistent with an emerging body of literature that supports protein multifunctionality under environmental stress. Collectively, this study contributes to the expanding field of plant-based heavy metal tolerance research. While many prior investigations have focused on well-studied model plants or hyperaccumulators, *Grewia asiatica* remains largely unexplored in this context. By integrating anatomical observations with molecular modelling and docking analysis, our work underscores the potential of this species in heavy metal-stressed environments and supports its further exploration for ecological restoration or green detoxification strategies.

Receptor	Ligand	Binding energy (Kj/mol)	Bond category	Bond type	Distance
RuBisCO	$HgCl_2$	-9.20	Electrostatic	Attractive	3.62
			Electrostatic bond	Attractive	3.57
			Other	Metal acceptor	2.46
			Unfavourable	Unfavourable Metal donor	2.12

Table 4: Molecular Interactions Between Hg^{2+} ion and RuBisCO Protein Showing Bond Category, Type, and Distance

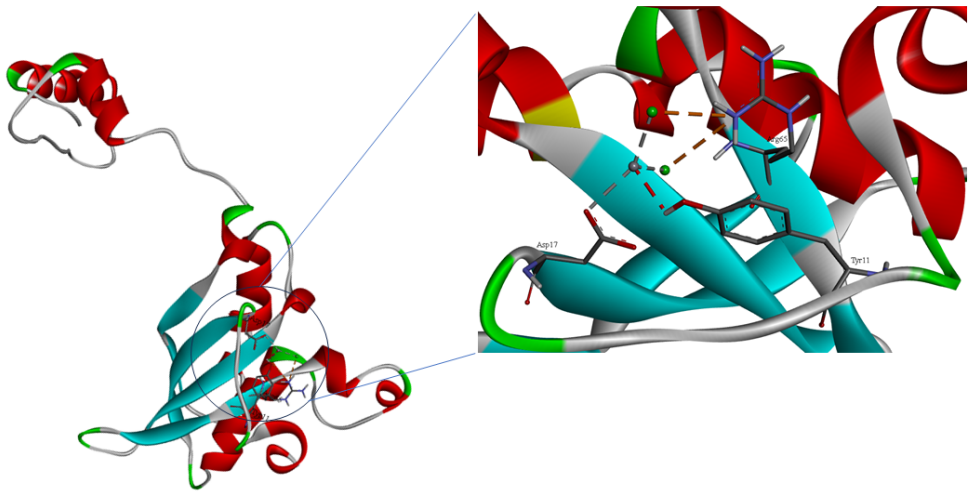

Figure 10a: 3D view of Molecular Interaction of RuBisCO with Hg^{2+}

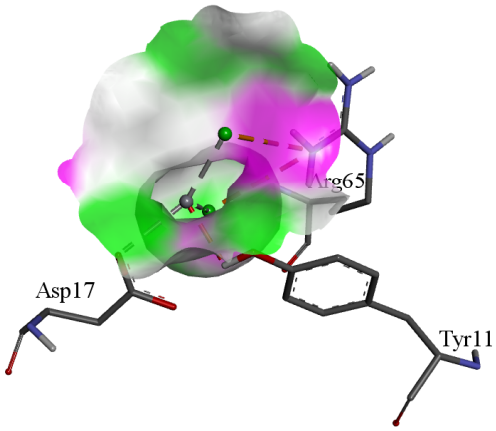

Figure 10b: Docking of Hg^{2+} ion with RuBisCO Showing key Interacting Residues within the Predicted Binding Pocket, Visualized Using Biovia Discovery Studio

Conclusion

This study demonstrates that *Grewia asiatica* L. exhibits both anatomical resilience and molecular adaptability when exposed to mercury stress. FESEM imaging confirmed substantial but non-lethal structural damage in roots and leaves, suggesting partial tolerance. More significantly, the bioinformatics investigation of RuBisCO revealed that this essential enzyme possesses structural features compatible with environmental stress endurance, including stability, solubility, and flexible secondary structures. The molecular docking results support the hypothesis that RuBisCO may serve as a metal-binding protein, potentially contributing to intracellular detoxification mechanisms. Such dual functionality metabolic and protective could represent an evolutionary adaptation in plants growing in metal-rich environments. These findings position *Grewia asiatica* as a promising candidate for ecological restoration and environmental clean-up in mercury-contaminated areas. However, for its application at scale, further studies are needed. Future work should focus on transcriptomic profiling of metal-responsive genes, quantification of mercury accumulation in plant tissues, and assessment of antioxidant enzyme activities. In addition, field trials are essential to validate the lab-scale findings and evaluate real-world efficacy. By integrating anatomical observations with protein-level insights, this study provides a holistic view of how *Grewia asiatica* responds to mercury exposure bridging cellular effects and molecular functions, and opening new avenues for its environmental application.

Acknowledgement

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Authors' Contributions

All authors have made significant contributions to the successful completion of this research. Dr. Sabyasachi Chatterjee conceptualized and supervised the overall study, providing critical guidance throughout the design and execution of the work. Mr. Swarup Mukherjee was primarily responsible for conducting the experimental work, including sample preparation, data collection, and initial analysis. Mr. Pritish Mitra played a pivotal role in performing and interpreting the bioinformatics and molecular docking studies, significantly contributing to the computational component of the research. The manuscript was jointly prepared by Swarup Mukherjee and Dr. Sabyasachi Chatterjee, with valuable inputs from Mr. Pritish Mitra in refining the bioinformatics content. All authors have read and approved the final version of the manuscript.

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