



Tissue Specific Genomic and Proteomic Profiling in Albino Wistar Rat

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Authors' contributions

This work was carried out in collaboration among all authors. Author RS conducted laboratory experiments, data collection, data curation, manuscript preparation, writing, review, and editing. Author TK was responsible for validation, visualization, and data curation. Authors SP, AS, Anirban Chakraborty, RS, IK, SM, TR, RP, RS, Arijit Chakraborty, MK, DP, MS, SM, SS, TN, DP and PR were involved in animal maintenance, laboratory experiments, and data collection. Author SMD contributed to supervision, conceptualization, visualization, validation, data curation, and manuscript preparation. All authors read and approved the final manuscript.

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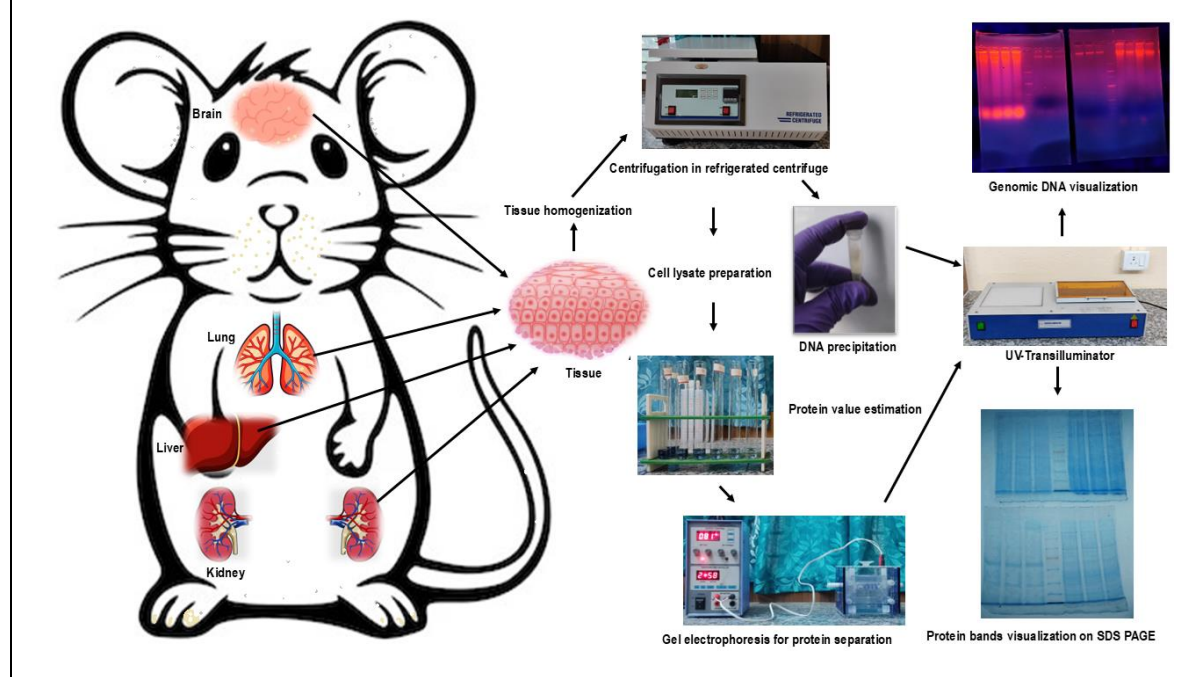
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Abstract

Animal tissues from Albino Wistar rats provide a valuable model to compare uniform genomic DNA with tissue-specific protein expression, enabling baseline molecular profiling across organs. This study aimed to isolate high-quality genomic DNA and characterize tissue-specific protein expression profiles from liver, kidney, lung, and brain tissues of albino Wistar rat (*Rattus norvegicus*). Genomic DNA was extracted using the phenol-chloroform method and evaluated by spectrophotometric analysis and agarose gel electrophoresis. All samples exhibited high purity (A260/A280: 1.80-1.97) and intact high-molecular-weight DNA bands with minimal degradation. Total protein was quantified using the Lowry method, demonstrating tissue-dependent variation, with the highest concentration observed in liver (93 µg/mL), followed by kidney (86 µg/mL), lung (78.6 µg/mL), and brain (76.5 µg/mL). SDS-PAGE analysis revealed distinct and complex banding patterns across tissues, reflecting differential protein expression profiles. Despite uniform genomic integrity across tissues, proteomic heterogeneity was evident, consistent with organ-specific functional specialization. This study provides baseline comparative molecular profiling across rat tissue and establishes reliable protocols for simultaneous genomic and proteomic analysis and provides baseline molecular data for future biomedical and toxicological investigations.



Keywords: *Rattus norvegicus*; genomic DNA isolation; protein estimation; SDS-PAGE; electrophoresis.

1. Introduction

Animal tissues are a rich source of biomolecules including nucleic acids and proteins, which serve as vital components for studying genetic and physiological responses (Yadav et al., 2021). Experimental animal models play a pivotal role in advancing molecular and biomedical research. Among them, the Albino Wistar rat (*Rattus norvegicus*) is extensively utilized due to its genetic stability, physiological resemblance to humans, and adaptability to controlled laboratory conditions (Krubaa & Yogitha, 2024; Olson, 2016; Prins et al., 2024). Molecular investigations using this model organism provide valuable insights into normal physiological processes as well as pathological conditions (Aitman et al., 2011; Saha, 2026; Saha & Dutta, 2025). At the molecular level, biological systems are governed by two fundamental macromolecules genomic DNA and proteins. Genomic DNA serves as the repository of hereditary information, maintaining structural consistency across different tissues of an organism (Table 1), whereas proteins represent the functional output of gene expression and exhibit dynamic, tissue-specific variability (Yadav et al., 2021; Dapic et al., 2019). Isolation of high-quality genomic DNA is a critical prerequisite for downstream applications such as polymerase chain reaction (PCR), sequencing, and gene expression analysis (Sirakov, 2016). Among various extraction

techniques, the phenol-chloroform method remains a widely accepted approach due to its efficiency in removing proteins and other contaminants, thereby yielding high-purity DNA (Gautam, 2022; Dowhan, 2012). The integrity and purity of isolated DNA are commonly assessed using spectrophotometric analysis and agarose gel electrophoresis (Green and Sambrook, 2019; Voytas, 2000). Proteomic analysis provides insights into functional diversity at the cellular level (Saha & Dutta, 2024; Saha et al., 2025). Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is a well-established technique that separates proteins based on molecular weight following denaturation, allowing visualization of tissue-specific protein expression patterns (Blancher and Cormick, 2012; Gallagher, 2012). When combined with quantitative protein estimation methods such as the Lowry assay, SDS-PAGE enables comprehensive analysis of protein abundance and distribution (Lowry et al., 1951; Waterborg, 2009). Understanding the relationship between genomic uniformity and proteomic diversity across different tissues is essential for establishing baseline molecular profiles in experimental animals (Chowdhury et al., 2017; Gauci et al., 2011). Such information is particularly important in toxicological, pharmacological, and physiological studies, where tissue-specific responses to environmental or chemical stressors are investigated. However, comparative baseline molecular data across multiple rat tissues remain limited. Therefore, the present study aims to isolate genomic DNA and perform SDS-PAGE-based protein profiling from liver, lung, kidney, and brain tissues of Albino Wistar rat (*Rattus norvegicus*) in order to evaluate tissue-specific variations in molecular composition and establish a standardized framework for comparative biomolecular analysis.

Table 1. Physiological roles of different organs in the Wistar rat

Organs	Physiological roles	References
Liver	The metabolic hub, rich in enzymes.	(Rui, 2014; Alamri, 2018)
Lungs	Involved in gas exchange and surfactant production.	(Bernhard, 2016)
Kidney	Specialized in filtration and ion transport.	(Hoenig & Hladik, 2018)
Brain	Focused on signal transduction and lipid-rich neural structures.	(Incontro et al., 2025)

2. Methods and Methodology

2.1 Experimental Animal and Sample Collection

This study was designed as a methodological standardization experiment. Here we took four healthy adult male Albino Wistar rats (*R. norvegicus*), weighing approximately 200gm $\pm$ 15gm, were used in this study. All experimental procedures were conducted in accordance with Institutional Animal Ethical Committee (IAEC) guidelines. The animals were sacrificed under ethical conditions, and vital organs including liver, lung, kidney and brain were carefully dissected. The tissues were washed in 0.1 M phosphate-buffered saline (PBS; containing NaH_2PO_4 and Na_2HPO_4) (pH 7.4) to remove blood and debris, and subsequently stored at -20°C until further processing.

2.2 Preparation of Tissue Homogenate

Tissue samples were thawed at 4°C and homogenized to obtain a 20% (w/v) homogenate in lysis buffer (pH 7.4). For protein extraction, tissues were homogenized in 0.1 M PBS containing protease inhibitors to prevent protein degradation. The homogenates were centrifuged at 12,000 rpm, and the supernatant was collected for further analysis (Waterborg, 2009).

2.3 Genomic DNA Isolation

Genomic DNA was isolated using the phenol-chloroform extraction method (Gautam, 2022). Tissue samples were lysed in a buffer containing Tris-HCl (pH 7.4), EDTA, and sodium dodecyl sulfate (SDS), followed by treatment with proteinase K to digest proteins and nucleases (Dowhan, 2012). An equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added to the lysate, followed by centrifugation to separate the aqueous and organic phases. The aqueous phase containing DNA was carefully transferred to a fresh tube. DNA was precipitated using cold ethanol in the presence of sodium acetate, washed with 70% ethanol, air-dried, and resuspended in nuclease-free water (He et al., 2022). DNA purity was assessed spectrophotometrically by measuring A260/A280 ratios (Mehraj et al., 2025).

2.4 Agarose gel Electrophoresis for DNA Analysis

The integrity of genomic DNA was evaluated using 1% agarose gel electrophoresis (Green & Sambrook, 2019). Agarose was dissolved in Tris-acetate-EDTA (TAE) buffer and stained with ethidium bromide. DNA samples were mixed with loading dye in 5:1 ratio and loaded into wells along with a DNA ladder. Electrophoresis was carried out at 50-80 V on the basis of gel size, and DNA bands were visualized under ultraviolet illumination using a Transilluminator (Sedlackova et al., 2013).

2.5 Protein Estimation

Protein concentration was determined using the Lowry method with bovine serum albumin (BSA) as the standard (Lowry et al., 1951). The assay is based on the reduction of Folin Ciocalteu reagent by copper-treated protein under alkaline conditions, resulting in a colorimetric change proportional to protein concentration (Waterborg, 2009). Absorbance was measured spectrophotometrically, and protein concentrations were calculated and result was expressed as $\mu\text{g}/\mu\text{l}$ using a BSA standard curve.

2.6 SDS PAGE for Protein Separation

Protein samples (50 $\mu\text{g}/\text{ml}$) were mixed with loading buffer containing SDS, β -mercaptoethanol, glycerol, and bromophenol blue and heated at 94°C for 5-6 minutes to ensure complete denaturation (Gallagher, 2012; Roy & Kumar, 2014). Samples were loaded onto a discontinuous polyacrylamide gel system consisting of stacking and resolving gels. Electrophoresis was performed under constant voltage, allowing proteins to separate based on molecular weight (Rath et al., 2013). Following electrophoresis, gels were stained with Coomassie Brilliant Blue, and protein bands were visualized. Molecular weights were estimated by comparison with a standard protein ladder.

3. Results

3.1 Genomic DNA Isolation and Quality Assessment

Genomic DNA extracted from all tissues exhibited high integrity and purity. Agarose gel electrophoresis showed distinct, high-molecular-weight bands localized near the wells, with negligible smearing, indicating minimal degradation (Fig. 1).

Spectrophotometric analysis revealed A260/A280 ratios ranging from 1.80 to 1.97 for 1 μl of DNA sample, confirming low protein contamination. Among the tissues, kidney samples demonstrated relatively higher purity (1.88-1.97), whereas liver, lung, kidney and brain exhibited consistent moderate yields with intact DNA (Table 2).

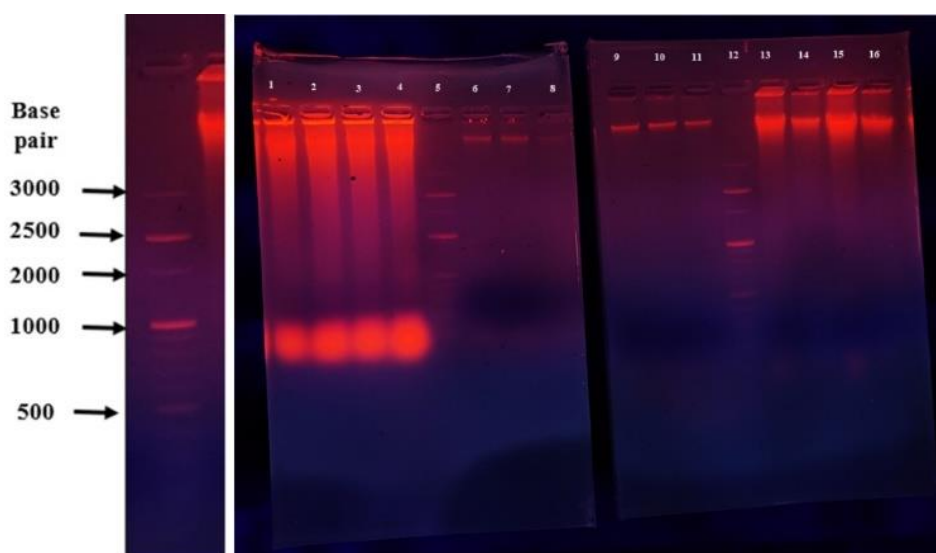


Fig. 1. Genomic DNA isolation from liver (lane 1,2,3,4), lung (lane 6,7,8), kidney (lane 9,10,11), and brain (lane 13,14,15,16) tissues of albino Wister rat, lane 5 and 12 are DNA ladders

Table 2. Genomic DNA yield and purity check

Organ	DNA Yield (µg/mg tissue)	A260/A280 Ratio	Integrity	References
Liver	Moderate	1.82-1.87	Intact	(Jang et al., 2022)
Lung	Moderate	1.80-1.88	Intact	(Niland et al., 2012)
Kidney	High	1.88-1.97	Intact	(Zheng et al., 2025)
Brain	Moderate	1.80-1.85	Intact	(Niland et al., 2012)

3.2 Protein Quantification Using BSA Standard Curve

The BSA standard curves showed strong linearity with a regression equation of $y = 0.0094x$ and a coefficient of determination $R^2 = 0.998$, confirming the accuracy and reliability of the assay. Liver tissues showed an estimated protein concentration of 93 µg/ml. Lung tissue showed an estimated protein concentration of approximately 78.6 µg/ml. Kidney tissues exhibited protein concentrations of approximately 86 µg/ml. Brain tissues showed estimated protein values of approximately 76.5 µg/ml. These quantitative results correlate well with the observed SDS-PAGE band intensities.

3.3 SDS-PAGE based Protein Profiling

SDS-PAGE analysis demonstrated successful separation of total proteins from all groups (Fig. 2). Multiple protein bands were observed across a wide molecular weight range, confirming efficient protein extraction and electrophoretic separation (Table 3).

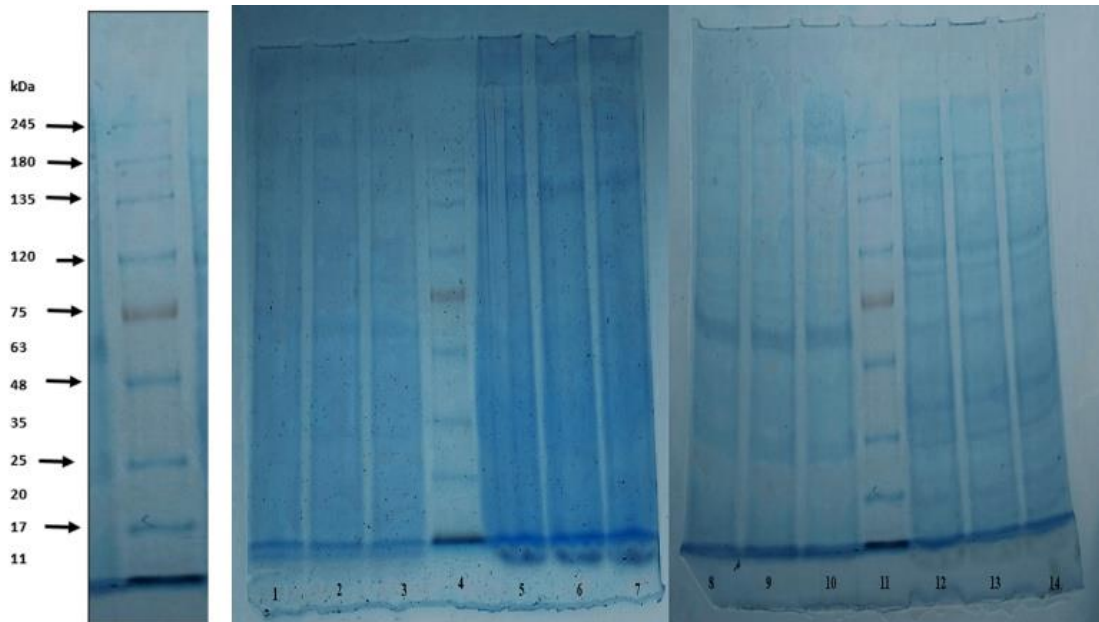


Fig. 2. Protein separation through SDS PAGE from liver (lane 1,2,3), lung (5,6,7), kidney (lane 8,9,10), and brain (12,13,14) tissues of albino Wister rat, lane 4 is a protein ladder

Table 3. Protein profiling through SDS PAGE from brain tissue of albino Wister rat

Molecular weight (kDa)	Proteins	Reference
~180	Myosin heavy chain, Spectrin	(Tikunov et al., 2001)
~135	RNA polymerase subunit, Cadherin fragments	(Goodwin et al., 2000)
~120	β-Catenin, Clathrin light chain	(Vondrak et al., 2024)

Molecular weight (kDa)	Proteins	Reference
~100	Heat shock protein 90 (HSP90), DNA topoisomerase I	(Csermely & Yahara, 2002)
~75	HSP70, Transferrin	(Wittrup et al., 2010)
~63	Serum albumin, Tubulin	(Maldonado et al., 2008)
~35	GAPDH, Carbonic anhydrase	(Roy et al., 2010)
~25	Glutathione S-transferase (GST), Cyclophilin	(Carrello et al., 2004)
~23	Superoxide dismutase (Cu/Zn-SOD), Peroxiredoxin	(Huang et al., 2006)
~17	Histones (H2A/H2B), Calmodulin	(Novosylna et al., 2017)

4. Discussion

The present study demonstrates the successful isolation of high-quality genomic DNA from multiple tissues of Albino Wistar rat using the phenol-chloroform method. The observed A260/A280 ratios (1.80-1.97) and the presence of intact high-molecular-weight bands with minimal smearing confirm the purity and integrity of the extracted DNA. These findings are consistent with earlier reports indicating that phenol-chloroform extraction yields DNA suitable for downstream molecular applications such as PCR and sequencing (Gautam, 2022; Green & Sambrook, 2019).

Although genomic DNA integrity remained consistent across all tissues, slight variations in yield and purity were observed. Kidney tissues exhibited relatively higher purity compared to other organs, which may be attributed to differences in cellular composition and lower lipid content. Similar observations have been reported in tissue-specific DNA extraction studies, where organ-specific biochemical composition influences extraction efficiency (He et al., 2022; Mehraj et al., 2025).

In contrast to the uniformity of genomic DNA, protein expression profiles showed significant tissue-specific variation. The liver exhibited the highest protein concentration, followed by kidney, lung, and brain tissues. This observation aligns with the well-established role of the liver as a major metabolic hub with high enzymatic activity, leading to increased protein abundance (Rui, 2014). Similarly, the kidney's involvement in filtration and transport processes contributes to its relatively high protein content (Hoenig & Hladik, 2018).

SDS-PAGE analysis revealed distinct banding patterns across tissues, reflecting differential protein expression and functional specialization. The presence of proteins such as heat shock proteins, cytoskeletal components, and metabolic enzymes corresponds with previously reported tissue-specific proteomic profiles (Dapic et al., 2019; Gallagher, 2012). The observed molecular weight distribution, including bands corresponding to HSP90, tubulin, and GAPDH, further supports the reliability of protein separation and identification. The strong correlation between spectrophotometric protein quantification and SDS-PAGE band intensity validates the consistency and accuracy of the experimental approach. The high linearity of the BSA standard curve ($R^2 = 0.998$) further confirms the reliability of the Lowry method for protein estimation (Waterborg, 2009).

Genomic DNA isolation and protein profiling through SDS PAGE constitute fundamental molecular approaches for assessing organismal responses to environmental stressors across diverse taxa. In avian systems, chronic exposure to air pollutants such as particulate matter, heavy metals, and polycyclic aromatic hydrocarbons induces genomic instability, reflected in altered DNA integrity and fragmentation patterns, alongside differential protein expression associated with oxidative stress, detoxification pathways, and immune modulation (Saha et al., 2025). Similarly, temperature-induced stress in zebra fish leads to perturbations in genomic stability and transcriptional regulation, which are subsequently manifested at the proteomic level through altered expression of heat shock proteins, metabolic enzymes, and antioxidant defense molecules detectable via SDS PAGE (Bag et al., 2026). In the context of Indian stray dogs, environmental stressors including thermal extremes and urban pollutants contribute to molecular adaptations involving DNA damage responses and shifts in serum and tissue protein profiles, indicating physiological strain and adaptive resilience (Saha et al., 2025). Furthermore, insect biodiversity, being highly sensitive to environmental fluctuations, exhibits significant variations in genomic and proteomic architecture under stress conditions, where DNA isolation reveals mutation or polymorphism patterns, and SDS PAGE highlights stress-responsive protein bands linked to survival and adaptation mechanisms (Saha et al., 2025). Collectively, the integration of genomic DNA isolation and protein separation

techniques provides a robust framework for elucidating molecular-level impacts of environmental stressors across ecological systems.

Overall, the study highlights a fundamental biological principle, while the genome remains structurally conserved across tissues, the proteome exhibits dynamic and tissue-specific variation driven by functional requirements. These findings are in agreement with previous studies emphasizing the role of differential protein expression in defining organ-specific physiological functions. The integration of genomic and proteomic analyses in this study provides a comprehensive molecular overview and establishes baseline data for future research in toxicology, pharmacology, and comparative physiology.

5. Conclusion

The study successfully demonstrated standardized protocols for genomic DNA isolation and SDS-PAGE protein profiling from four rat tissues. Obtained biomolecules were intact and suitable for advanced molecular analysis. This study validates the fundamental principle of molecular biology, that a single genome provides the instructions for a diverse and specialized proteome. The successful extraction and profiling of these macromolecules provide a reliable reference for future studies in toxico-genomics or gene expression using the Wistar rat as a model organism. The high purity of the DNA and the clear resolution of the protein bands confirm that the methods employed were robust and reproducible. This study successfully demonstrates the isolation of high-quality genomic DNA and the characterization of tissue-specific protein profiles in Albino Wistar rat tissues. The combined use of agarose gel electrophoresis, SDS-PAGE, and spectrophotometric protein estimation confirms the reliability of the experimental approach and highlights molecular diversity across tissues. Tissue heterogeneity represents a significant confounding factor, as each organ comprises multiple cell types with distinct molecular profiles. Bulk tissue analysis therefore masks cell-specific variations, limiting resolution at the cellular or subcellular level. The present study successfully established reliable protocols for genomic DNA isolation and SDS PAGE based protein profiling in Albino Wistar rat tissues. These findings provide a reliable molecular baseline and methodological framework for future investigations in toxicology, gene expression studies, and comparative physiology using Wistar rat as a model organism.

6. Highlights of this study

- Rats exhibit strong physiological and metabolic homology with humans, enabling translational relevance of organ-specific proteomic and genomic findings.
- The well-annotated genome of rats facilitates accurate correlation between DNA integrity and tissue-specific protein expression.
- Adequate tissue yield in rats allows simultaneous high-quality extraction of proteins and genomic DNA across multiple organs.
- Rat organ systems display sensitive and distinct biochemical responses, supporting precise detection of tissue-specific protein profiles.
- Established experimental reliability, ease of handling, and standardized ethical protocols make rats a consistent and reproducible model organism.

Ethical Approval

The Institutional Animal Ethical Committee of Midnapore City College approved the animal experiments in Central Animal Facility, Midnapore City College (2256/PO/Re/S/23/CCSEA).

Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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Competing Interests

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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