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Microscopic Investigation and Identification of Mitotic Stages Using Onion (*Allium cepa*) and Onion Spring (*Allium fistulosum*)

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ABSTRACT

Mitosis is a fundamental process in plant growth and development because it governs the production of new cells in meristematic tissues. Examining its stages provides insights into genetic stability and cellular behavior, which are relevant to agriculture, crop improvement, and environmental monitoring. Although cytological studies using *Allium* species are well established, there is continued value in demonstrating how classical staining methods, such as acetocarmine, effectively visualize mitotic events in educational and basic laboratory settings. This study aimed to identify and describe the stages of mitosis in onion (*Allium cepa*) and spring onion (*Allium fistulosum*) root tips using acetocarmine-stained squash preparations. Fresh root tips were hydrolyzed in hydrochloric acid, stained with acetocarmine, and examined under a compound light microscope. The observed mitotic stages were documented based on chromatin morphology and nuclear characteristics. Both species exhibited similar observable mitotic features under the staining and microscopic conditions used, with clear visualization of prophase, metaphase, and telophase, identified by chromatin condensation, chromosome alignment, and nuclear reformation, respectively. Anaphase was not observed, likely due to its brief duration relative to the other phases. Overall, the results demonstrate that acetocarmine staining provides reliable

visualization of major mitotic stages**,** although it lacks sufficient resolution to distinguish individual chromosomes. These findings confirm that simple cytological techniques remain effective tools for demonstrating mitosis in instructional and introductory research contexts. Future studies may employ DNA-specific stains, digital imaging, and larger sample sizes to capture short-lived stages and enable quantitative analysis.

Keywords: *mitosis, Allium cepa, Allium fistulosum, cytological techniques, acetocarmine staining*

Introduction

Mitosis is a fundamental biological mechanism that ensures the accurate segregation of genetic material during cell division, playing a critical role in plant development, tissue differentiation, and genomic stability. In higher plants, meristematic cells of the root tip provide an ideal system for studying mitotic stages because of their high rate of cell division and simple tissue organization (Sablowski & Gutierrez, 2022). Investigating mitosis is not only significant for understanding plant physiology but also for its applications in agriculture, environmental monitoring, and cytogenetic research (Robinson, 2021). For instance, the TCP15 transcription factor is highly expressed in young leaf primordia and actively promotes the mitotic cell cycle to drive leaf cell proliferation (Ding et al., 2022).

Among plant systems used in cytological research, species of the genus *Allium* are widely favored for their large, easily observable chromosomes and the simplicity of root tip preparation. The inclusion of *Allium fistulosum* provides additional observational context and helps demonstrate whether commonly described mitotic features in *Allium cepa* are also observable in a closely related species under similar laboratory conditions. The onion (*Allium cepa*) is a classic model in mitotic studies and is commonly used in genotoxicity assays such as the *Allium* test due to its chromosomal clarity and high sensitivity to external agents (Desvoyes et al., 2021; Liman et al., 2025). In addition, plant meristems—responsible for generating all organs—are vital targets in crop improvement strategies (Lindsay et al., 2024). This study also includes *Allium fistulosum* (spring onion), which is morphologically distinct from *A. cepa* due to its stalk-producing habit and lack of a true bulb. The inclusion of *A. fistulosum* allows for comparative analysis and highlights whether cytological characteristics observed in *A. cepa* are consistent across closely related species. Although no observable differences in mitotic phases were detected between the two, their morphological contrast provides pedagogical value in educational contexts.

Standard cytological procedures, including fixation of root tips using Carnoy's solution, acid hydrolysis, and subsequent DNA staining with Feulgen or aceto-orcein, enable clear visualization of condensed chromosomes under the light microscope (Desvoyes et al., 2021; Ramirez-Castillo et al., 2024; Ristea & Zarnescu, 2024). Previous studies have demonstrated the importance of microscopic and laboratory-based analyses in understanding plant structure, health, and growth responses. For instance, histological examination has been used to evaluate tissue-level changes in plants affected by pathogens, highlighting the role of microscopy in plant research and

diagnostics (Biol & Juruena, 2024). In addition, experimental studies on crop growth and development have emphasized the value of laboratory-based plant investigations in supporting agricultural research and instruction (Cablinan, 2024). These studies support the relevance of microscopic techniques in plant science and provide a local research context for the present cytological investigation of mitosis in *Allium* species. Squash preparations of the stained root meristems facilitate the identification of distinct mitotic stages: prophase, metaphase, anaphase, and telophase. While modern fluorochromes offer advanced resolution, traditional stains like acetocarmine remain cost-effective, accessible, and instructional for both research and teaching purposes. Despite these advantages, there are limited descriptive cytological studies that document mitotic features in more than one *Allium* species using standardized laboratory procedures. This study addresses this gap by documenting and describing observable mitotic stages in *Allium cepa* and *Allium fistulosum* root tips using acetocarmine staining. Specifically, the objectives of this study were to prepare and stain root tips of *Allium cepa* and *Allium fistulosum* using acetocarmine and to identify and describe the observable features of the different mitotic stages under a compound light microscope.

Methods

Research Design

This study employed a descriptive–experimental research design, specifically using qualitative visual observations to examine the stages of mitosis in onion (*Allium cepa*) and spring onion (*Allium fistulosum*) root tips. The descriptive component involved documenting the visible characteristics of each mitotic phase under the microscope, while the experimental component consisted of preparing root-tip squashes through hydrolysis and staining to enhance chromosome visibility. This design was appropriate because it enabled systematic examination of mitotic activity while utilizing laboratory procedures that clarified cellular structures.

Locale of the Study

The study was conducted on July 22, 2025, at the Science Laboratory of Monkayo College of Arts, Sciences, and Technology (MONCAST), Monkayo, Davao de Oro, Philippines. The laboratory provided controlled conditions and access to essential equipment such as compound microscopes, glassware, and reagents necessary for cytological specimen preparation.

Research Instruments

The instruments used in the study included compound light microscopes (40x magnification), clean glass slides, cover slips, scalpels, and Petri dishes, which were necessary for specimen preparation and microscopic observation. Reagents used were 1N hydrochloric acid (HCl) for hydrolysis and 2-5% acetocarmine solution for staining. These instruments and chemicals were selected for their suitability in preparing clear mitotic squashes and ensuring accurate identification of chromatin structures.

Data Collection Procedures

Fresh bulbs of onion and spring onion were placed in water with only the basal portion submerged and allowed to develop roots for 24 hours under ambient laboratory conditions. Once roots reached 1–2 cm, they were excised using a clean scalpel. The cut

root tips were placed in a Petri dish containing 1 N HCl and hydrolyzed at 60 °C for exactly 6 minutes to soften cell walls and facilitate staining.

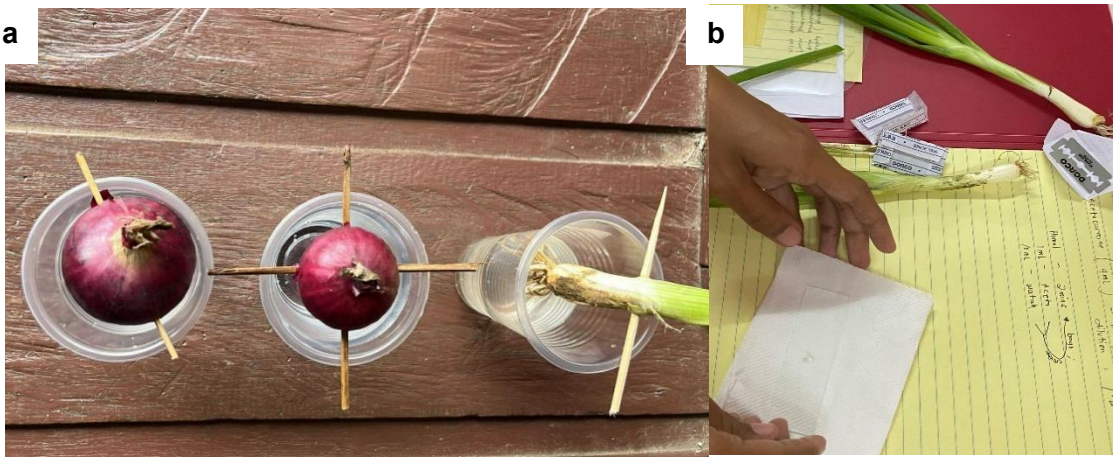
Analysis of Data

Data were analyzed qualitatively through descriptive visual observation of chromatin and nuclear features corresponding to each mitotic stage. Observed nuclear and chromatin features were compared with established cytological descriptions from related literature to ensure accuracy. No statistical treatment was applied because the study focused on qualitative morphological characteristics rather than quantitative measurements.

Preparation of the Specimen

Fresh bulbs of onion and spring onion were placed in water, with only the basal portion submerged, and were allowed to sprout roots in ambient conditions for 24 hours. Once roots reached a length of approximately 1–2 cm, they were excised using a clean scalpel or blade. The cut root tips were then immediately transferred into a clean petri dish containing a few milliliters of 1 M hydrochloric acid (HCl) and hydrolyzed at 60 °C for exactly 6 minutes to soften cell walls and facilitate staining. This acid treatment helped to soften the cell walls by breaking down the middle lamella, facilitating easier staining and slide preparation (Parrott Lab, 2024). The specimens were left in the HCl solution for five minutes before proceeding to staining.

The overall workflow for specimen preparation, from root sprouting to staining, is summarized in Figure 1.



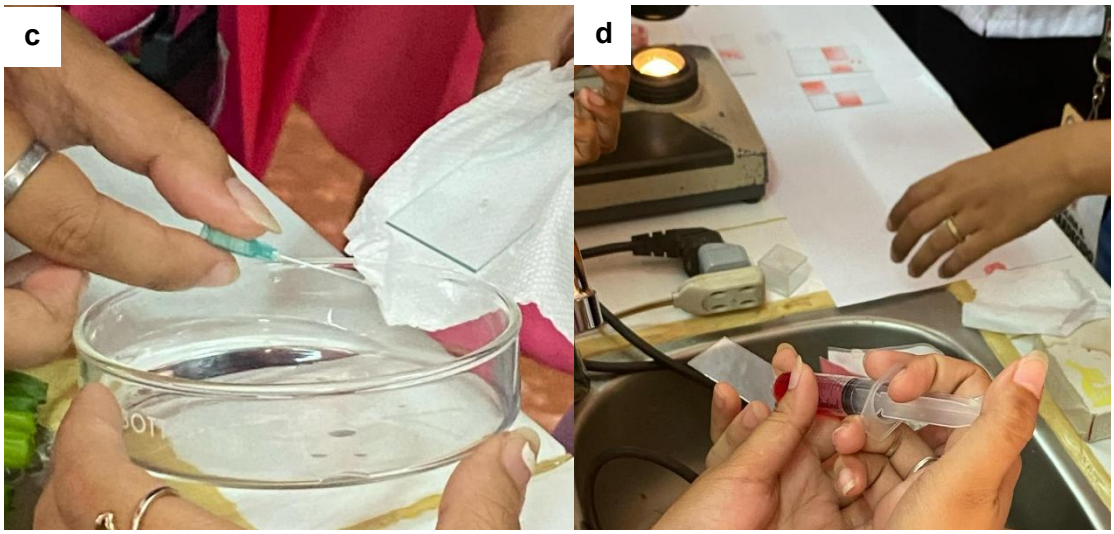


Figure 1. *Preparation of Specimens: a) Root Basal Soaking for Roots to Sprout; b) Excising Newly Sprouted Roots Using Blade; c) Soaking Excised Roots in Hydrochloric Acid (HCl) in a Petri Dish; and d) Subjecting Spring Onion Roots to Acetocarmine Solution*

Preparation of the Materials

Before slide preparation, all materials were assembled, including clean slides and cover slips, a compound microscope, a scalpel, forceps, HCl, acetocarmine stain, and droppers for transferring reagents. Ensuring that all materials were clean and readily accessible allowed efficient preparation of high-quality squash mounts. Clean and manageable materials (e.g., smudge-free slides) will facilitate smooth preparation of the squashes (Padmasri et al., 2025).

Procedure of the Chemical Agents

Actively growing onion root tips, approximately 1–2 mm in length, were selected because this region contains a high density of actively dividing meristematic cells (Sabeen et al., 2020). The roots were thoroughly rinsed and then hydrolyzed in 1 N hydrochloric acid (HCl) at 60 °C for 5–8 minutes, which helps soften the cell walls and facilitates cell separation during slide preparation (Ali et al., 2025). The softened root tips were then transferred to a clean microscope slide, where they were stained with acetocarmine solution at a concentration of 2–5%, which specifically binds to chromosomal DNA and allows for detailed visualization of mitotic chromosomes (Ristea & Zarnescu, 2023). The stained root tip was covered with a clean coverslip and gently squashed using thumb pressure or the flat end of a pencil eraser, which spreads the cells into a single layer for optimal viewing (Arora et al., 2023).

Microscopic Examination Procedures

Each prepared slide was examined under a compound light microscope. Initial scanning was performed using the 40× objective to locate regions with actively dividing meristematic cells. Detailed observations were made using the 100× oil-immersion objective (1000× magnification). Contrast adjustments were applied using the condenser and diaphragm.

To improve clarity, observations for each mitotic stage were organized into sub-paragraphs:

1. Prophase: Chromatin appeared as dense, darkly stained aggregates; the nucleolus was no longer visible.
2. Metaphase: Chromatin masses aligned at the cell's equatorial region, forming a distinct plate-like arrangement.
3. Anaphase: Two chromatin groups are separated and migrate toward opposite poles of the cell.
4. Telophase: Two re-forming nuclei appeared at opposite ends, with chromatin beginning to decondense.

Due to the non-specific nature of acetocarmine staining, individual chromosomes were not distinctly visible. Phase identification was based solely on chromatin distribution and overall nuclear morphology, consistent with observations from Ramirez-Castillo et al. (2024).

Ethical Considerations

The study was conducted in accordance with ethical laboratory practices, ensuring safety and integrity throughout the experiment. No human or animal subjects were involved, and only onion and spring onion root tips were used as specimens, minimizing ethical concerns regarding biological material. Laboratory reagents such as hydrochloric acid and acetocarmine were handled with care to avoid hazards, and proper disposal procedures were observed to prevent environmental harm. Academic honesty was also maintained by properly acknowledging sources of information and presenting observations truthfully without fabrication, thereby upholding the ethical standards of both research and scientific reporting.

Results and Discussion

Microscopic Examination

Microscopic examination of acetocarmine-stained meristematic cells of onion (*Allium cepa*) and spring onion (*Allium fistulosum*) root tips enables clear visualization of cells at each phase of mitosis (Ristea & Zarnescu, 2024). In prophase, the diffuse nuclear chromatin condenses into distinct, stainable chromosomes as the cell begins preparing for division (Iemura et al., 2021). During metaphase, the fully condensed chromosomes become aligned along the cell's equatorial plane (the metaphase plate) under the tension of spindle fibers (Manjula et al., 2024). In anaphase, the paired sister chromatids separate and are drawn toward opposite spindle poles, effectively segregating the genetic material (Konečná et al., 2023). Finally, in telophase, two new nuclear envelopes form around each set of separated chromosomes, and the chromosomes begin to decondense back into chromatin (Park et al., 2019). These phases produce characteristic, distinguishable figures under the light microscope with 40x magnification, and acetocarmine staining greatly enhances the contrast of chromosomal structures at each stage, facilitating their identification. Observations were qualitative and focused on identifying characteristic chromatin and nuclear features of each mitotic stage rather than quantifying their frequency.

The following sections describe the microscopic characteristics of each observed phase.

Prophase

In the acetocarmine-stained root tip preparations of *Allium cepa* and *A. fistulosum*, prophase cells were identified by their dense, dark red nuclei, which appeared smaller and more compact than those in interphase. This visual shift reflects

the early condensation of chromatin, which begins in prophase as the chromatin fibers coil tightly, leading to a reduction in nuclear size and the disappearance of the nucleolus (Ristea & Zarnescu, 2024). Although individual chromosomes could not be resolved due to the non-specific nature of acetocarmine, the intense nuclear staining provided sufficient contrast to distinguish prophase from interphase (Zhong et al., 2025). These observations are consistent with recent studies that describe acetocarmine as effective for highlighting overall nuclear morphology, even though it lacks the resolution of DNA-specific stains such as Feulgen or DAPI (Ramirez-Castillo et al., 2024).

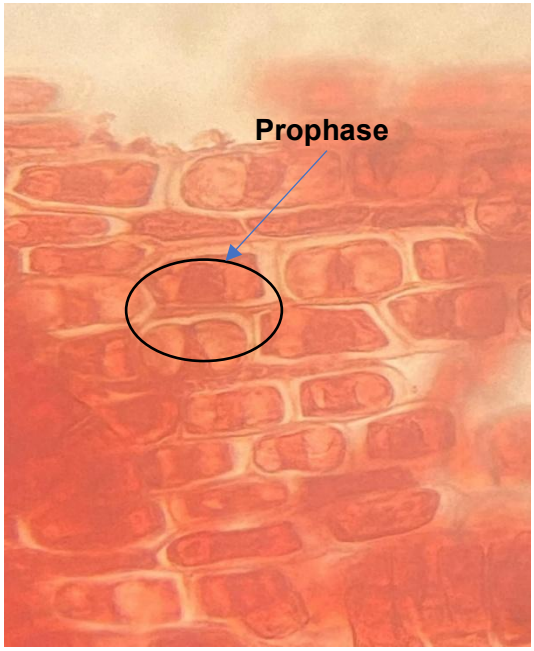


Figure 2. *Prophase as the First Stage of Mitosis*

Metaphase

Metaphase cells were seen as those with a single, intensely stained chromatin mass at the cell's equator. Acetocarmine binds DNA, so during metaphase – when the nuclear envelope has broken down and all chromosomes are highly condensed – the cell's central region appears as a dark, compact "plate" of chromatin rather than a distinct round nucleus. In practice, we identified metaphase by the loss of a visible nuclear membrane or nucleolus and the presence of this dense equatorial band of stain. This matches expectations from other Allium studies: for example, Animasaun et al. (2024) noted that colchicine treatment produces "a large number of metaphase cells" with very condensed chromosomes, while Küçük and Liman (2018) explicitly scored Allium root cells by mitotic phase (including metaphase) when assessing genotoxic effects. Since *A. fistulosum* (Welsh or "spring" onion) is a close relative of *A. cepa*, essentially identical metaphase figures in both species were observed. Thus, the root-tip squash showed that metaphase cells in both onions are recognized by a uniform, darkly stained chromatin mass aligned at mid-cell – reflecting the condensed chromosomes on the metaphase plate – in contrast to the larger, paler nuclei of interphase or the separated chromatin of anaphase. These observations are consistent with standard cytogenetic descriptions of plant mitosis and confirm that metaphase was

correctly identified by its characteristic nuclear morphology (dense, centrally located chromatin with no intact nuclear envelope).

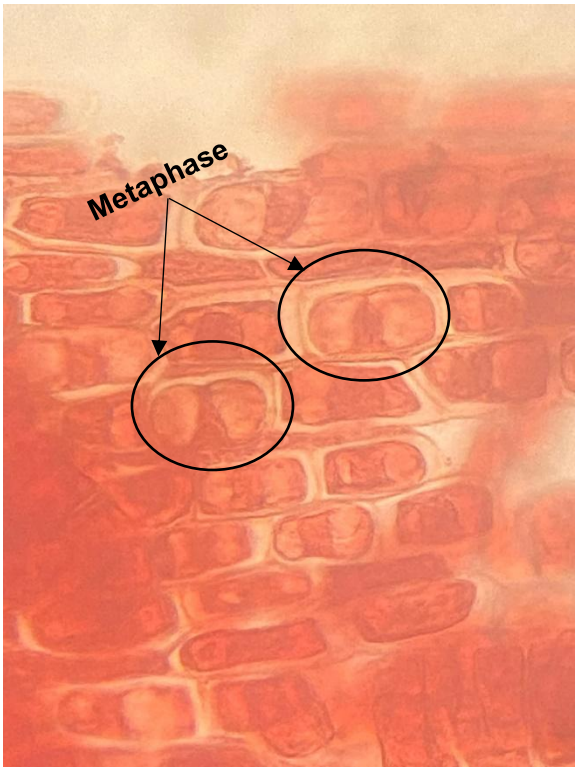


Figure 3. *Metaphase as the Second Stage of Mitosis*

Telophase

In both *Allium cepa* and *A. fistulosum* root-tip squash preparations stained with acetocarmine, telophase cells were identified by the formation of two distinct, round nuclei at opposite poles of the cell, signaling the reassembly of nuclear envelopes around the decondensing chromatin (Stamatiou & Vagnarelli, 2021). Although acetocarmine does not visualize individual chromosomes at high resolution, the appearance of two separated chromatin masses, each resembling interphase nuclei, marks the completion of mitosis (Onisan et al., 2025). Occasionally, a cell plate or division furrow was observed between the two nuclei, further indicating the final stages of cytokinesis (Cavusoglu, 2023). These visual criteria—chromatin decondensation, nuclear envelope reformation, and clear polar localization—are consistent with standard descriptions of telophase morphology in Allium root tips prepared using DNA-specific stains.

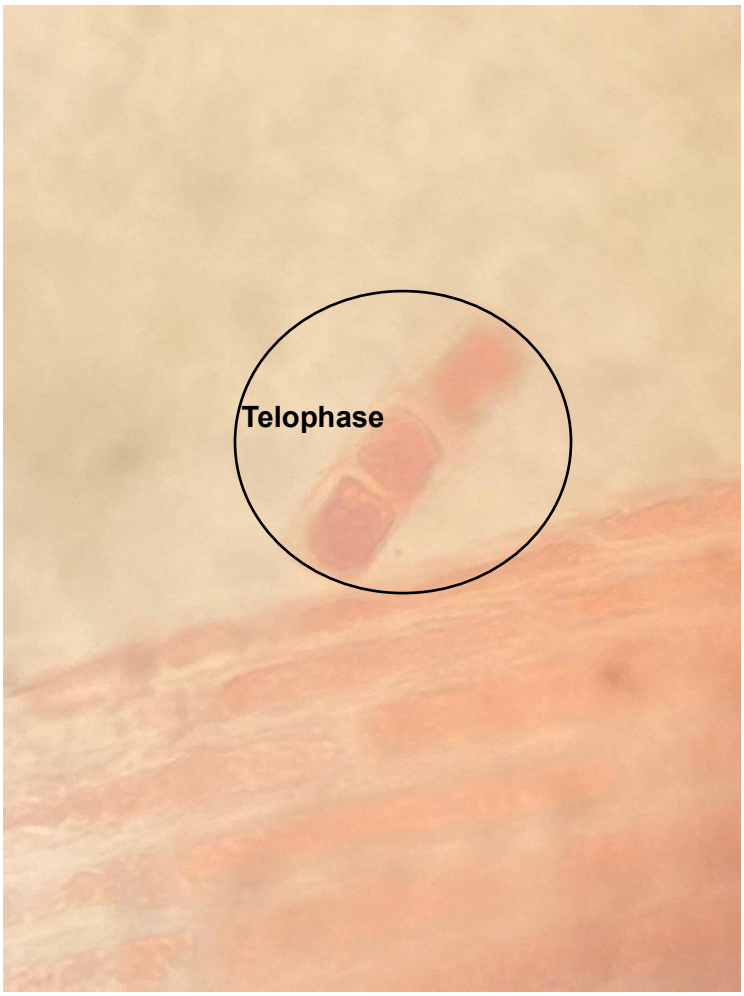


Figure 4. *Sample of a Telophase Cell*

Anaphase figures were not observed in the prepared slides, likely due to the brief duration of this stage.

Conclusion and Future Works

This study reinforces the value of classical cytological techniques in demonstrating fundamental cellular processes in plant biology. By confirming the suitability of acetocarmine-stained squash preparations for instructional and introductory research settings, the findings support their continued use in plant cytogenetics education and basic laboratory investigations. The results confirm that simple cytological techniques remain effective for visualizing major mitotic stages in instructional and basic research settings. However, anaphase was not observed in the examined specimens, likely due to its brief duration relative to other mitotic phases and the limited number of observable cells.

These findings support the continued use of classical staining methods in teaching laboratories and introductory cytogenetic studies. They also highlight the importance of methodological refinements to improve visualization of short-lived mitotic stages.

Future studies may consider using DNA-specific stains such as Feulgen or DAPI to enhance chromosome resolution and increasing sample size and number of prepared slides to capture transient stages, such as anaphase**.** Likewise, future researchers may consider applying digital imaging and image analysis tools to enable semi-quantitative assessment of mitotic activity and exploring the effects of environmental or chemical factors on mitosis in plant root meristems.

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Conflict of Interest

The author declares that there is no conflict of interest in the conduct of this study or in the preparation of this report. No financial or personal relationships with any individuals or organizations influenced the results or conclusions of this research.

Artificial Intelligence (AI) Declaration Statement

This study utilized artificial intelligence (AI) tools to support specific stages of the research development process. The primary AI tools used were ChatGPT by OpenAI and other common AI-based utilities such as grammar checkers and reference managers (e.g., Grammarly and Mendeley's AI-assisted citation suggestions). These tools were employed solely for non-analytical tasks, including generating initial drafts of explanations, refining the clarity of sentences, assisting in the organization of ideas, formatting citations, and checking grammar and coherence. No AI tool was used to generate, manipulate, or fabricate data, nor to conduct analyses, statistical computations, or conclusions. The extent of AI use was limited to the following activities:

1. Drafting and improving narrative sections such as background information, related literature summaries, and methodological descriptions.

2. Assisting in language enhancement, consistency checking, and proper formatting based on journal requirements.
3. Helping identify potential structure improvements, topic transitions, and paraphrasing overly complex sentences for readability.

A verification process was strictly followed to ensure the accuracy, integrity, and reliability of all AI-assisted content. All AI-generated or AI-refined text underwent a comprehensive manual review by the author. This included the following:

1. Cross-checking all content against primary sources, research data, and authoritative references.
2. Editing or rewriting segments where AI responses lacked precision, contained generic statements, or required technical specificity.
3. Ensuring that interpretations, scientific claims, and conceptual explanations reflected the authors' own analysis and expertise.
4. Running plagiarism checks to confirm originality and compliance with academic standards.

The authors take full responsibility for all content presented in this manuscript and affirm that the intellectual conclusions, analyses, and interpretations are entirely their own.