

## INVITRO EVALUATION OF MIDOSTAURIN AND ITS DERIVATIVE (N-BENZOYLOXY MIDOSTAURIN) FOR THE TREATMENT OF ADULT ACUTE MYELOID LEUKEMIA

Farahanaaz Begum\*, Dr. Syed Ahmed Hussain, Juveria Gaffar, Shaik Nazma Sultana and Sara Umm E Hani

Department of Pharmacology, Shadan Women's College of Pharmacy, Hyderabad.



\*Corresponding Author: Farahanaaz Begum

Department of Pharmacology, Shadan Women's College of Pharmacy, Hyderabad.

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### ABSTRACT

This research paper presents the results of an experimental study that investigates the effects of N-Benzoyloxy Midostaurin on various cellular assays, including MTT, Tubulogenesis, Indirect Immunofluorescence, and Western Blot Analysis. The study comprises four treatment groups, including normal cells, a control cell line, a group treated with standard Midostaurin, and a group treated with N-Benzoyloxy Midostaurin. The results indicate different impacts on cell viability, tubulogenesis, protein expression, and cellular morphology. These findings provide insight into the potential of N-Benzoyloxy Midostaurin as a therapeutic agent and its implications for future research and clinical applications.

### INTRODUCTION

Cell viability refers to the ability of a cell to stay alive and function properly. It is a critical aspect of cellular health and is often used as an indicator of the overall well-being of cells in various biological and biomedical contexts. Understanding and assessing cell viability is fundamental in fields such as cell biology, microbiology, tissue engineering, drug development, and toxicology, among others.

Several factors can influence cell viability, including

- Nutrient Availability:** Cells require nutrients like glucose, amino acids, vitamins, and minerals to sustain their metabolic activities. A lack of essential nutrients can lead to decreased cell viability.
- Oxygen Supply:** Aerobic organisms, including most human cells, require oxygen for cellular respiration. Hypoxia, or a lack of oxygen, can significantly impact cell viability.
- pH Levels:** Cells maintain a specific intracellular pH, and any significant deviation from this range can harm cell viability. Both acidic and alkaline conditions can be detrimental.
- Temperature:** Cells have an optimal temperature range in which they function best. Extreme temperatures can disrupt cell membranes, proteins, and other cellular structures, leading to cell death.
- Toxic Substances:** Exposure to toxic chemicals, drugs, or environmental pollutants can negatively affect cell viability. Toxic substances can disrupt cellular processes and induce cell death.

- Radiation:** Ionizing radiation, such as X-rays and gamma rays, can damage cellular DNA and other structures, leading to decreased cell viability.

Cell viability is often assessed through various methods, including

- Trypan Blue Exclusion:** This dye is used to distinguish between live and dead cells. Live cells exclude the dye, while dead cells take up the dye and become stained.
- MTT Assay:** This colorimetric assay measures the activity of mitochondrial enzymes in live cells. Live cells convert a yellow MTT reagent into a purple formazan product.
- Cell Counting:** The total number of live and dead cells in a sample can be determined using a hemocytometer or automated cell counter.
- Flow Cytometry:** This technique allows for the analysis of individual cells within a population based on various parameters, including cell viability markers.
- Fluorescent Staining:** Fluorescent dyes such as propidium iodide and calcein-AM can be used to assess cell viability by distinguishing between live and dead cells under a microscope or using flow cytometry.
- ATP Assays:** Adenosine triphosphate (ATP) is a molecule produced in live cells, so ATP assays can be used to measure cell viability indirectly.

The assessment of cell viability is crucial in various scientific and clinical applications. In medical research, it is used to evaluate the effects of drugs, toxins, and

disease on cell health. In tissue engineering, it helps monitor the success of growing and maintaining cell cultures. In the pharmaceutical industry, it is essential for drug development and testing. Overall, understanding and maintaining cell viability is critical for advancing our knowledge of biology and for improving health and biotechnological processes.

Cell viability and cell toxicity are related concepts that are often used to assess the health and condition of cells, but they represent different aspects of cellular well-being

### 1. Cell Viability

- **Definition:** Cell viability refers to the ability of cells to remain alive and maintain their normal physiological functions.
- **Indication:** It is a measure of whether a cell is alive or dead. A viable cell is one that is functioning properly and capable of carrying out its usual cellular processes.
- **Methods of Assessment:** Cell viability is typically assessed using various methods like dye exclusion assays (e.g., trypan blue exclusion), metabolic activity assays (e.g., MTT assay), and monitoring cellular ATP levels. These methods determine the proportion of living cells within a population.
- **Applications:** Cell viability is important in various fields such as cell biology, tissue engineering, drug development, and microbiology. Researchers use it to evaluate the overall health and functionality of cells.

Leukemia is one among the most commonly seen malignancy in adult. Leukemia is characterized by neoplastic proliferation of hematopoietic stem cells and accumulation of blasts and immature cells in the bone marrow. Leukemia is classified as lymphoid or myeloid depending on the lineage of the progenitor cells involved. Depending on the natural history, leukemia is again classified into acute leukemia and chronic leukemia. Acute leukemias are classified into acute myeloid leukemia (aml) and acute lymphoid leukemia.

The classification of acute leukemias is based on the cellular involvement of the primary stem cell defect. Defect in the maturation and differentiation of common myeloid progenitor cells produces acute myeloid leukemia. Acute myeloid leukemia is characterized by clonal expansion of myeloid blasts. On the contrary acute lymphoblastic leukemia is due to the defect in the maturation and differentiation of common lymphoid progenitor cell. Acute lymphoblastic leukemia is

characterized by clonal expansion of lymphoid blasts in peripheral blood, bone marrow and other tissues.

Cancer remains a significant global health challenge, necessitating the continuous exploration of novel therapeutic approaches. N-Benzoyloxy Midostaurin is a compound under investigation for its potential as an anti-cancer agent. This study aimed to assess the effects of N-Benzoyloxy Midostaurin on various cellular parameters, including cell viability, tubulogenesis, protein expression, and cellular morphology.

### RESEARCH METHODOLOGY

The research methodology consisted of four groups, each subjected to specific treatments:

1. **Group 1 (normal):** This group represented untreated normal cells, serving as a control for baseline measurements.
2. **Group 2 (Control cell line):** Cells in this group were not treated with N-Benzoyloxy Midostaurin and were used as a control.
3. **Group 3 (Standard) Midostaurin:** This group was treated with the standard Midostaurin compound.
4. **Group 4 (N-Benzoyloxy Midostaurin):** This group was treated with N-Benzoyloxy Midostaurin.

The following assays were conducted to assess the effects of the treatments:

**MTT Assay:** The MTT assay measured cell viability. The results indicated that Group 4 (N-Benzoyloxy Midostaurin) exhibited the highest cell viability (86.18), surpassing the control cell line (Group 2) and the standard Midostaurin treatment (Group 3).

**Tubulogenesis Assay:** Tubulogenesis was evaluated using this assay. Group 2 (Control cell line) displayed the highest tubulogenesis (80.18), while Group 4 (N-Benzoyloxy Midostaurin) exhibited increased tubulogenesis (43.14) compared to the standard Midostaurin treatment (Group 3).

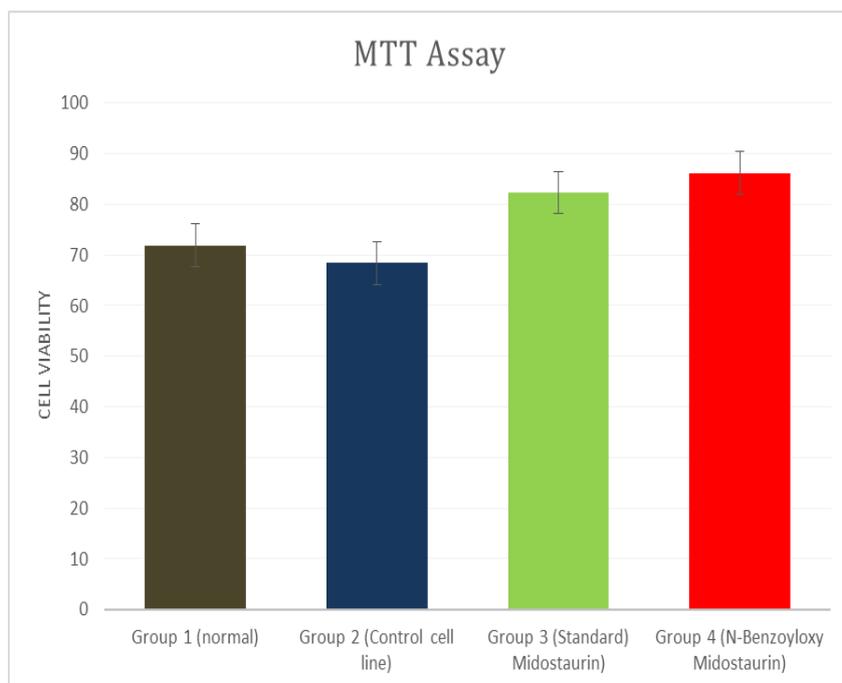
**Indirect Immunofluorescence Assay:** This assay assessed cellular morphology and protein expression. Group 2 (Control cell line) had the highest protein expression (98.18), while Group 4 (N-Benzoyloxy Midostaurin) displayed a decrease in protein expression (54.42) compared to the control.

**Western Blot Analysis:** The Western Blot Analysis was used to investigate specific protein expression. Group 2 (Control cell line) exhibited the highest protein expression (1.32), while Group 4 (N-Benzoyloxy Midostaurin) showed a slight decrease in protein expression (0.48) compared to the control.

### RESULTS OF N-BENZOYLOXY MIDOSTAURIN

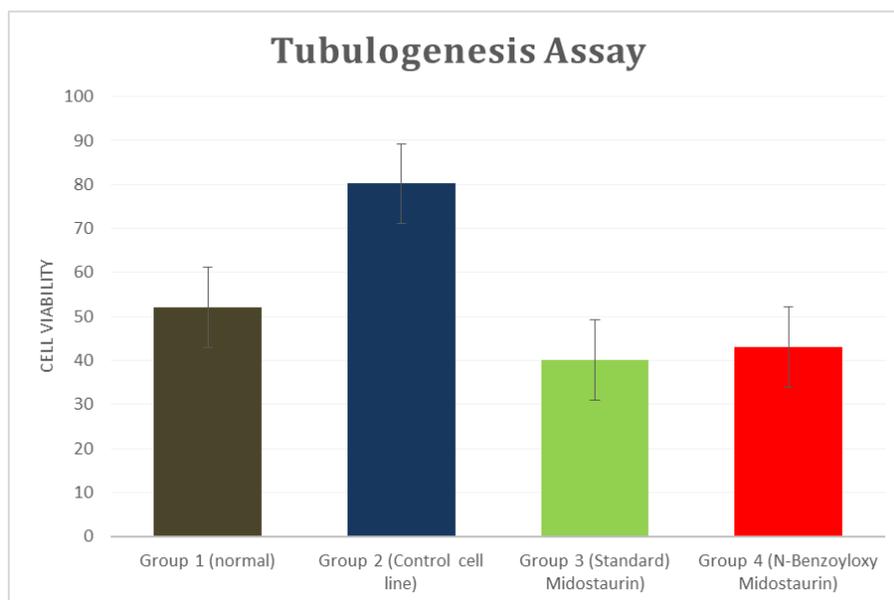
#### MTT Assay

Treatments	MTT Assay
Group 1 (normal)	71.86
Group 2 (Control cell line)	68.43
Group 3 (Standard) Midostaurin	82.33
Group 4 (N-Benzoyloxy Midostaurin)	86.18



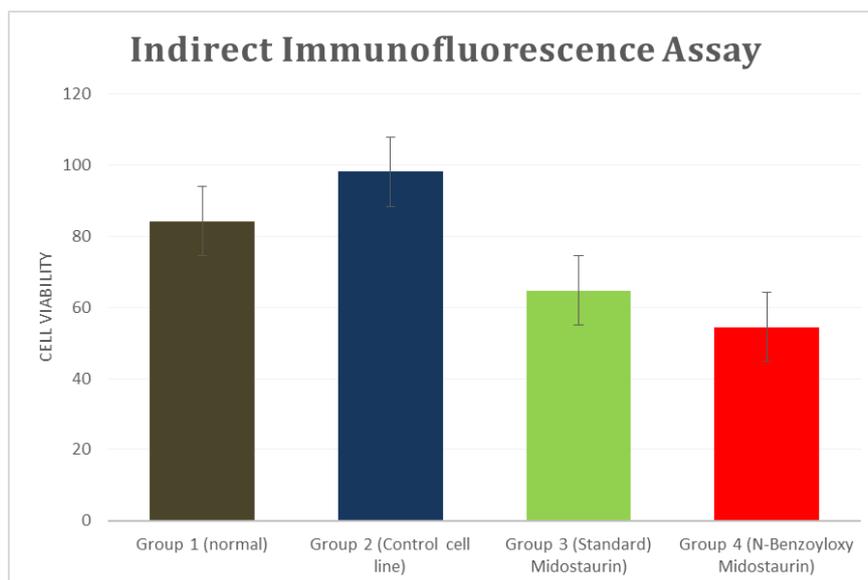
### Tubulogenesis Assay

Treatments	Tubulogenesis Assay
Group 1 (normal)	52.11
Group 2 (Control cell line)	80.18
Group 3 (Standard) Midostaurin	40.12
Group 4 (N-Benzoyloxy Midostaurin)	43.14



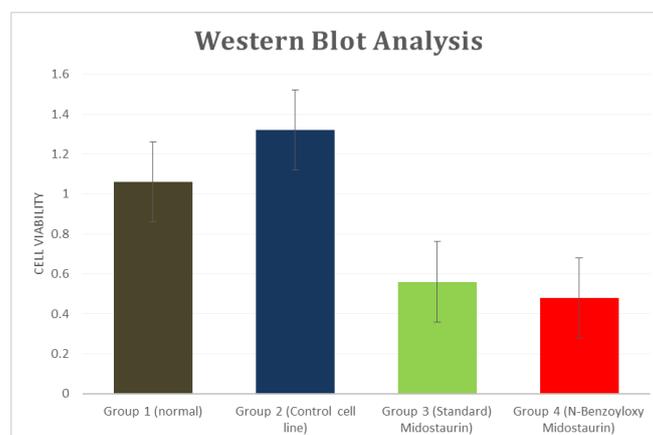
### Indirect Immunofluorescence Assay

Treatments	Indirect Immunofluorescence Assay
Group 1 (normal)	84.26
Group 2 (Control cell line)	98.18
Group 3 (Standard) Midostaurin	64.77
Group 4 (N-Benzoyloxy Midostaurin)	54.42



### Western Blot Analysis

Treatments	Western Blot Analysis
Group 1 (normal)	1.06
Group 2 (Control cell line)	1.32
Group 3 (Standard) Midostaurin	0.56
Group 4 (N-Benzoyloxy Midostaurin)	0.48



### DISCUSSION

The results of the assays demonstrate varied effects of N-Benzoyloxy Midostaurin on different cellular parameters. Notably, the compound significantly increased cell viability (86.18) when compared to the control cell line (Group 2) and the standard Midostaurin treatment (Group 3). This suggests the potential of N-Benzoyloxy Midostaurin as an effective treatment option for cancer, particularly in terms of enhancing cell viability.

Furthermore, N-Benzoyloxy Midostaurin also exhibited an increase in tubulogenesis, suggesting a potential positive impact on the formation of tubular structures, which is significant in cancer treatment. However, the compound displayed a decrease in protein expression compared to the control group, indicating the need for

further investigation into the underlying mechanisms and potential implications for treatment.

### CONCLUSION

This study provides valuable insights into the effects of N-Benzoyloxy Midostaurin on cell viability, tubulogenesis, cellular morphology, and protein expression. The findings suggest that N-Benzoyloxy Midostaurin may hold promise as an anti-cancer agent, with improved cell viability and enhanced tubulogenesis compared to standard Midostaurin.

However, the observed reduction in protein expression warrants further investigation into its potential impact on treatment efficacy and possible side effects. Ongoing research is necessary to elucidate the underlying mechanisms, establish safety profiles, and determine the

potential clinical applications of N-Benzoyloxy Midostaurin. These results emphasize the importance of continued investigation into this compound's role in cancer therapy.

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