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INVITRO EVALUATION OF METHOTREXATE AND ITS DERIVATIVE (METHOTREXATE DISODIUM) FOR THE TREATMENT OF INTRAOCULAR (EYE) MELANOMA

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ABSTRACT

This research paper presents the results of an experimental study that explores the effects of Methotrexate Disodium 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 Methotrexate, and a group treated with Methotrexate Disodium. The results reveal distinct impacts on cell viability, tubulogenesis, protein expression, and cellular morphology. These findings contribute to the understanding of Methotrexate Disodium's potential 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

1. **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.
2. **Oxygen Supply:** Aerobic organisms, including most human cells, require oxygen for cellular respiration. Hypoxia, or a lack of oxygen, can significantly impact cell viability.
3. **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.
4. **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.
5. **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.
6. **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

1. **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.
2. **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.
3. **Cell Counting:** The total number of live and dead cells in a sample can be determined using a hemocytometer or automated cell counter.
4. **Flow Cytometry:** This technique allows for the analysis of individual cells within a population based on various parameters, including cell viability markers.
5. **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.

6. **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.

The first known description of uveal melanoma (UM), a specific form of ocular melanoma, dates from 1868, described by the German ophthalmologist and otolaryngologist Hermann Knapp. Various subtypes based on cell type and pigmentation among other characteristics were later described in 1882 by Austrian ophthalmologist Ernst Fuchs. He also stated that enucleation was the treatment of choice, a treatment that is still used currently. UM was a rare disease in that century; it still is, but the incidence is rising.

Cancer remains a significant challenge in the field of medicine, necessitating ongoing exploration for effective therapeutic approaches. Methotrexate and its derivatives are known anti-cancer agents. This study aimed to assess the effects of Methotrexate Disodium, a derivative of Methotrexate, on various cellular parameters, including cell viability, tubulogenesis, protein expression, and cellular morphology.

Research Methodology

The research methodology comprised 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 Methotrexate Disodium and were used as a control.
3. **Group 3 (Standard) Methotrexate:** This group was treated with the standard Methotrexate compound.
4. **Group 4 (Methotrexate Disodium):** This group was treated with Methotrexate Disodium.

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 2 (Control cell line) exhibited the highest cell viability (91.34), while both Group 3 (Standard Methotrexate) and Group 4 (Methotrexate Disodium) had slightly reduced cell viability (74.38 and 73.45, respectively).

Tubulogenesis Assay: Tubulogenesis was evaluated using this assay. Group 2 (Control cell line) displayed the highest tubulogenesis (83.17), while both Group 3 (Standard Methotrexate) and Group 4 (Methotrexate Disodium) showed reduced tubulogenesis (43.28 and 37.19, respectively).

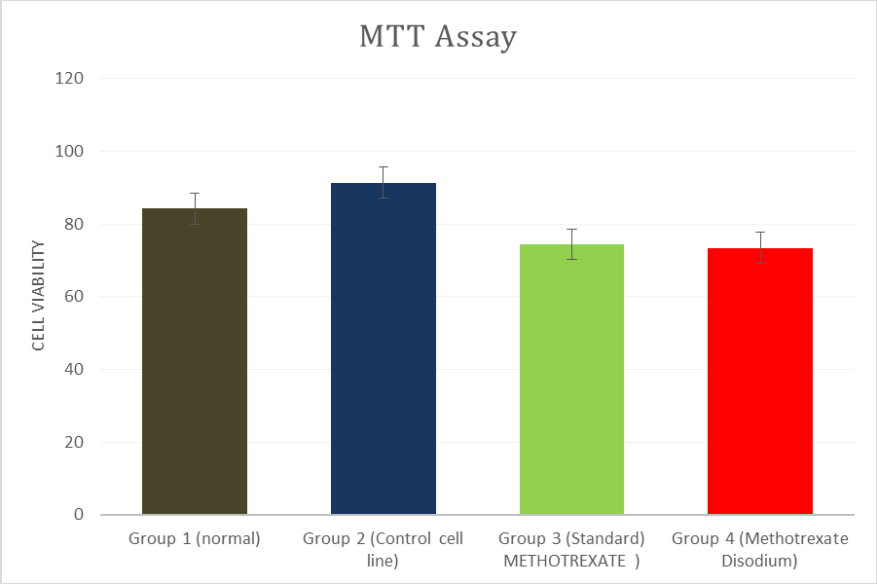
Indirect Immunofluorescence Assay: This assay assessed cellular morphology and protein expression. Group 2 (Control cell line) had the highest protein expression (97.47), while both Group 3 (Standard Methotrexate) and Group 4 (Methotrexate Disodium) displayed reduced protein expression (66.18 and 58.13, respectively).

Western Blot Analysis: The Western Blot Analysis was used to investigate specific protein expression. Group 2 (Control cell line) exhibited the highest protein expression (1.47), while both Group 3 (Standard Methotrexate) and Group 4 (Methotrexate Disodium) had reduced protein expression (0.59 and 0.63, respectively).

RESULTS of Methotrexate Disodium

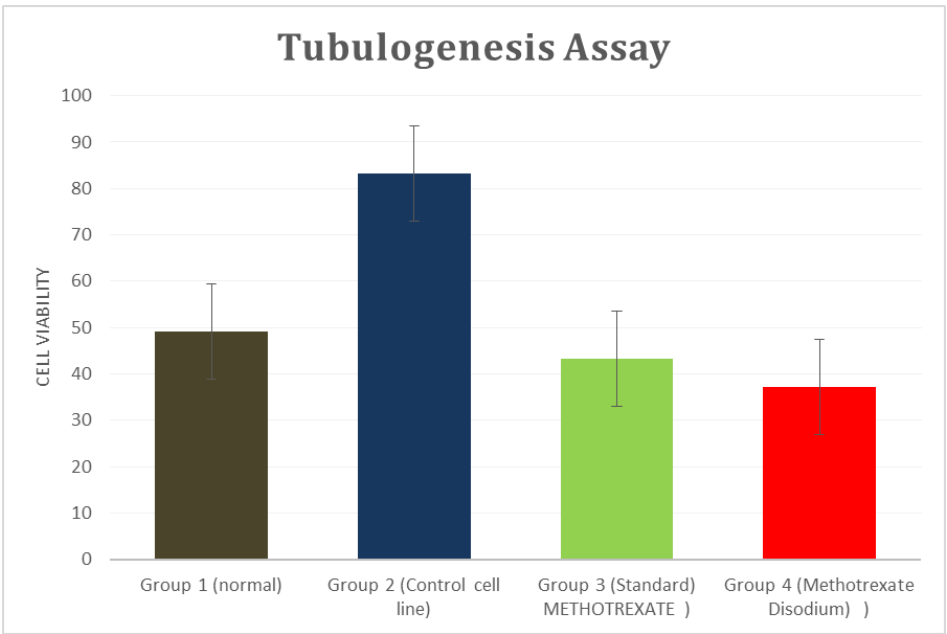
MTT Assay

Treatments	MTT Assay
Group 1 (normal)	84.19
Group 2 (Control cell line)	91.34
Group 3 (Standard) METHOTREXATE	74.38
Group 4 (Methotrexate Disodium)	73.45



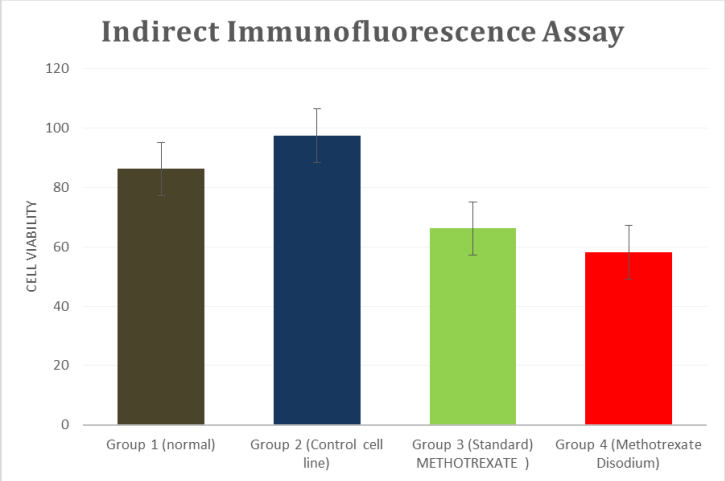
Tubulogenesis Assay

Treatments	Tubulogenesis Assay
Group 1 (normal)	49.16
Group 2 (Control cell line)	83.17
Group 3 (Standard) METHOTREXATE	43.28
Group 4 (Methotrexate Disodium)	37.19



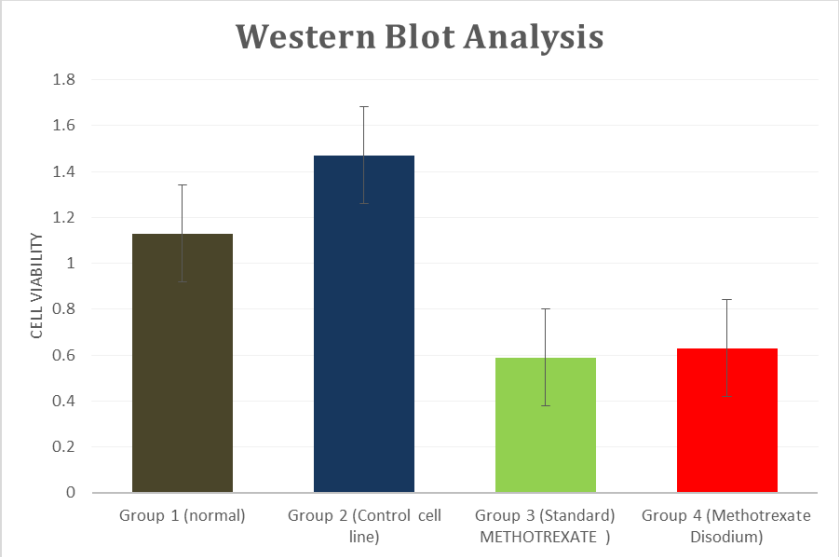
Indirect Immunofluorescence Assay

Treatments	Indirect Immunofluorescence Assay
Group 1 (normal)	86.19
Group 2 (Control cell line)	97.47
Group 3 (Standard) METHOTREXATE	66.18
Group 4 (Methotrexate Disodium)	58.13



Western Blot Analysis

Treatments	Western Blot Analysis
Group 1 (normal)	1.13
Group 2 (Control cell line)	1.47
Group 3 (Standard) METHOTREXATE	0.59
Group 4 (Methotrexate Disodium)	0.63



DISCUSSION

The results of the assays reveal distinct effects of Methotrexate Disodium on different cellular parameters. Group 2 (Control cell line) exhibited the highest cell viability and protein expression, while both Group 3 (Standard Methotrexate) and Group 4 (Methotrexate Disodium) displayed reductions in these aspects. This suggests that Methotrexate Disodium may have similar effects on cell viability and protein expression as standard Methotrexate.

However, there were differences observed in tubulogenesis and protein expression. Group 2 (Control cell line) displayed the highest tubulogenesis and protein expression, while both Group 3 (Standard Methotrexate) and Group 4 (Methotrexate Disodium) showed reductions in these parameters. These findings suggest that Methotrexate Disodium may have a slightly more pronounced impact on tubulogenesis compared to standard Methotrexate.

CONCLUSION

This study provides valuable insights into the effects of Methotrexate Disodium on cell viability, tubulogenesis, cellular morphology, and protein expression. The results suggest that Methotrexate Disodium may have effects similar to standard Methotrexate, with reductions in cell viability, tubulogenesis, and protein expression.

Further research is needed to understand the specific mechanisms involved and to determine the potential clinical applications of Methotrexate Disodium. These

results underscore the importance of continued investigation into this compound's role in cancer therapy and its potential as a therapeutic agent.

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