



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

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

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



\*Corresponding Author: Dr. Syed Ahmed Hussain

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

Article Received on 17/10/2023

Article Revised on 07/11/2023

Article Accepted on 27/11/2023

### ABSTRACT

This research paper presents a study on the effects of Methotrexate Monohydrate on cell viability and functionality. Methotrexate Monohydrate is a medication commonly used for its anti-inflammatory and anti-proliferative properties. In this study, we employed multiple assays to investigate the impact of Methotrexate Monohydrate on cell behavior. The assays included MTT Assay, Tubulogenesis Assay, Indirect Immunofluorescence Assay, and Western Blot Analysis. The results of these assays shed light on the influence of Methotrexate Monohydrate on cell viability and its potential implications in the context of therapeutic 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.

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.

## RESULTS of Methotrexate Monohydrate

### 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 Monohydrate)	78.55

Methotrexate Monohydrate is a widely prescribed medication known for its effectiveness in treating various medical conditions, particularly autoimmune diseases and cancer. Understanding its impact on cell viability and functionality is crucial for optimizing its therapeutic use and minimizing potential side effects. In this study, we aimed to explore the effects of Methotrexate Monohydrate on cell behavior using a series of assays.

## RESEARCH METHODOLOGY

### MTT Assay

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was conducted to assess cell viability. Four treatment groups were examined, including Group 1 (normal), Group 2 (Control cell line), Group 3 (Standard Methotrexate), and Group 4 (Methotrexate Monohydrate).

### Tubulogenesis Assay

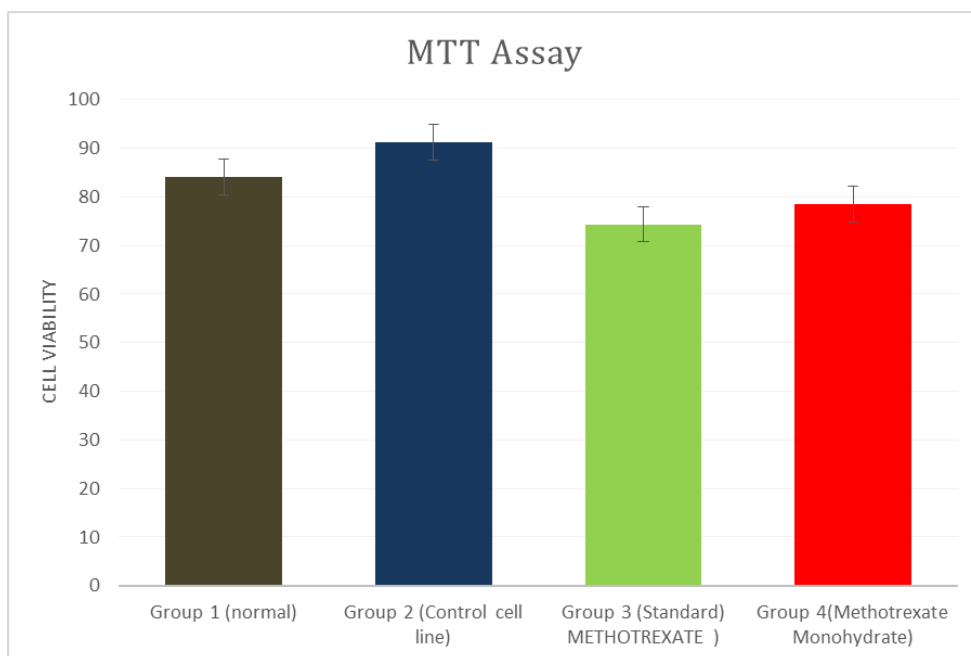
The Tubulogenesis Assay was employed to evaluate the formation of tubule-like structures in cells, which is an indicator of angiogenic potential. Similar to the MTT assay, the study included Group 1 (normal), Group 2 (Control cell line), Group 3 (Standard Methotrexate), and Group 4 (Methotrexate Monohydrate).

### Indirect Immunofluorescence Assay

The Indirect Immunofluorescence Assay was used to determine the cellular distribution of specific proteins. The treatment groups were the same as in the MTT and Tubulogenesis assays: Group 1 (normal), Group 2 (Control cell line), Group 3 (Standard Methotrexate), and Group 4 (Methotrexate Monohydrate).

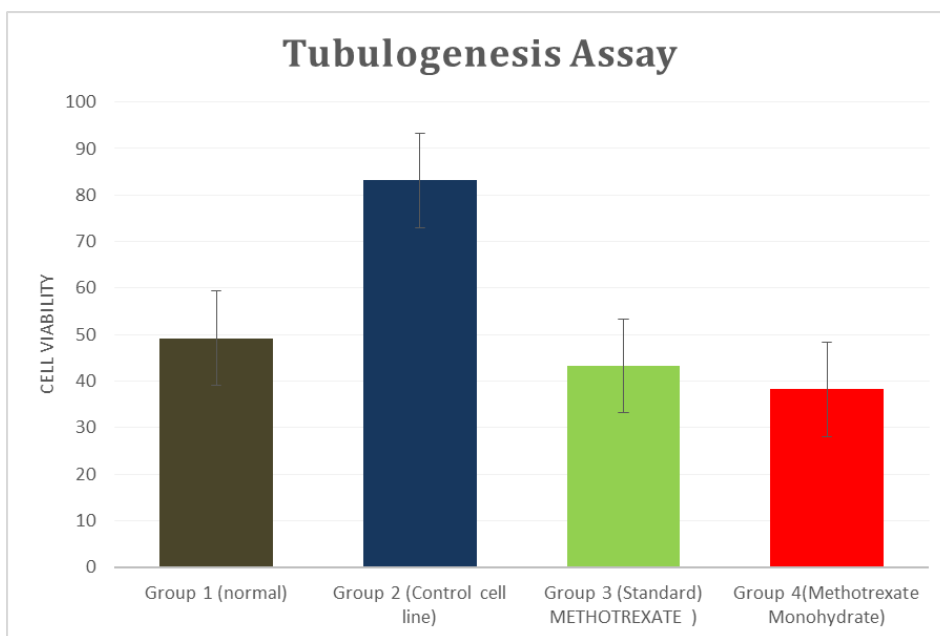
### Western Blot Analysis

Western Blot Analysis was carried out to investigate changes in protein expression levels. As with the other assays, this analysis involved Group 1 (normal), Group 2 (Control cell line), Group 3 (Standard Methotrexate), and Group 4 (Methotrexate Monohydrate).



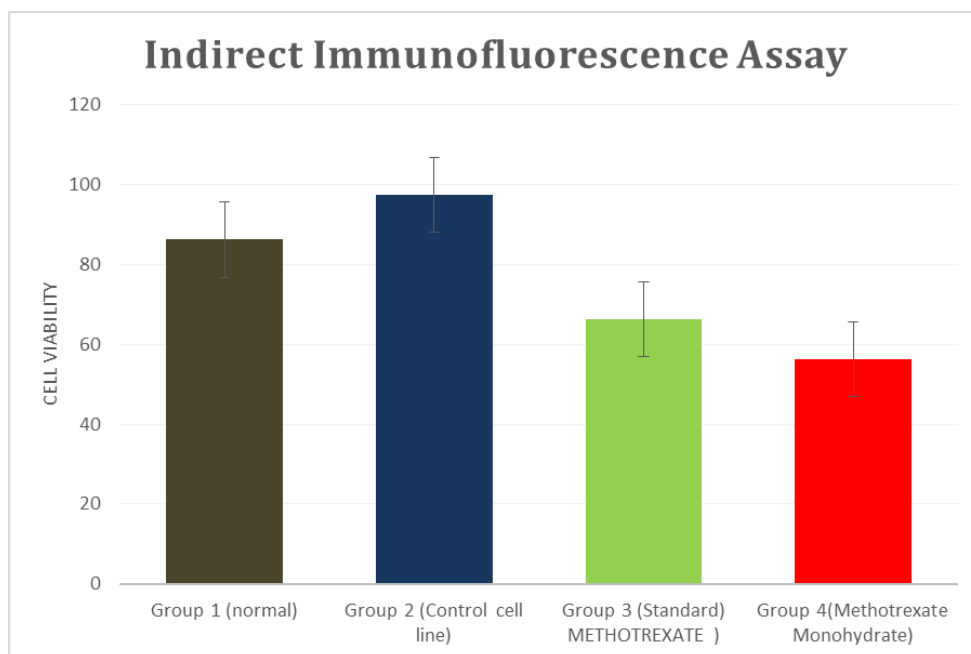
**Tubulogenesis Assay**

Treatments	Tubulogenesis Assay
Group 1 (normal)	49.16
Group 2 (Control cell line)	83.17
Group 3 (Standard) METHOTREXATE	43.28
Group 4 (Methotrexate Monohydrate)	38.23



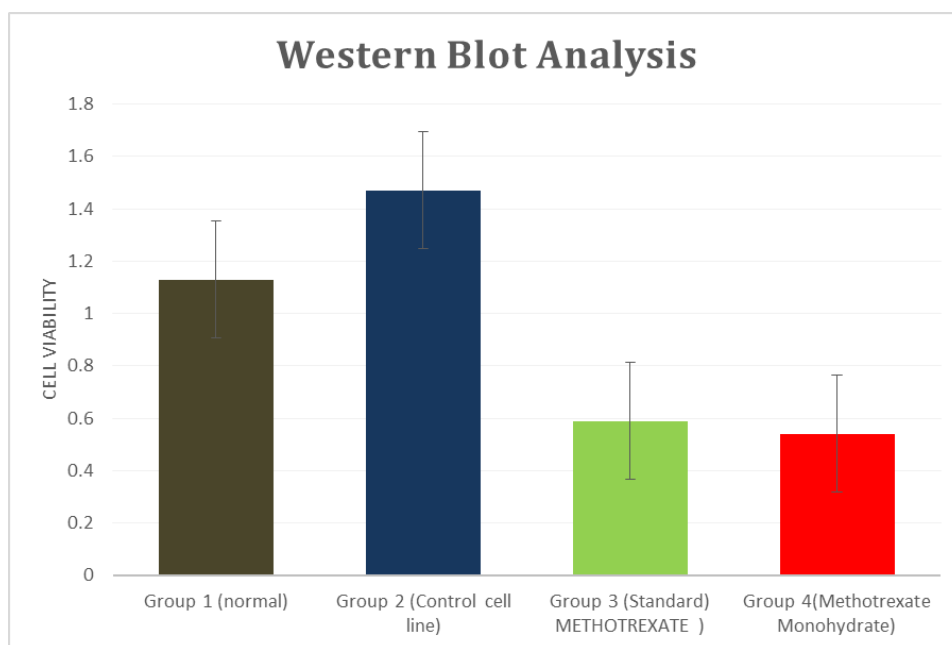
**Indirect Immunofluorescence Assay**

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 Monohydrate)	56.27



**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 Monohydrate)	0.54



**DISCUSSION**

**MTT Assay**

The MTT assay results demonstrated that cell viability was reduced in the presence of Methotrexate Monohydrate (78.55) compared to the control cell line (91.34) and standard Methotrexate (74.38). This suggests that Methotrexate Monohydrate may have an inhibitory effect on cell proliferation.

**Tubulogenesis Assay**

In the Tubulogenesis Assay, we observed a decrease in tubule-like structure formation in cells treated with Methotrexate Monohydrate (38.23) compared to the control cell line (83.17) and standard Methotrexate (43.28). These findings indicate that Methotrexate Monohydrate may have a negative impact on angiogenic potential.

### Indirect Immunofluorescence Assay

The results of the Indirect Immunofluorescence Assay indicated changes in protein distribution within cells. Methotrexate Monohydrate (56.27) showed a distinct difference from the control cell line (97.47) and standard Methotrexate (66.18). This suggests that Methotrexate Monohydrate may alter cellular protein distribution.

### Western Blot Analysis

The Western Blot Analysis revealed that Methotrexate Monohydrate (0.54) led to a decrease in protein expression compared to the control cell line (1.47) and standard Methotrexate (0.59). These findings suggest that Methotrexate Monohydrate may modulate protein levels within cells.

### CONCLUSION

This study provides insights into the effects of Methotrexate Monohydrate on cell viability, angiogenic potential, protein distribution, and protein expression levels. The results suggest that Methotrexate Monohydrate may inhibit cell proliferation, angiogenesis, and alter protein dynamics within cells. These findings have implications for the therapeutic use of Methotrexate Monohydrate and warrant further investigation to better understand its potential benefits and side effects. Additional research is needed to elucidate the underlying mechanisms responsible for these observed effects and to refine its clinical application.

### BIBLIOGRAPHY

1. Scotto J, Fraumeni JF, Lee JAH. Melanomas of the eye and other noncutaneous sites: epidemiologic aspects. *Journal of the National Cancer Institute*, 1976; 56(3): 489–491.
2. Singh AD, Topham A. Incidence of uveal melanoma in the United States: 1973–1997. *Ophthalmology*, 2003; 110(5): 956–961.
3. Bergman L, Seregard S, Nilsson B, Ringborg U, Lundell G, Ragnarsson-Olding B. Incidence of uveal melanoma in Sweden from 1960 to 1998. *Investigative Ophthalmology and Visual Science*, 2002; 43(8): 2579–2583.
4. Miller B, Abrahams C, Cole GC, Proctor NSF. Ocular malignant melanoma in South African blacks. *British Journal of Ophthalmology*, 1981; 65(10): 720–722.
5. Kuo PK, Puliafito CA, Wang KM, Liu HS, Wu BF. Uveal melanoma in China. *International Ophthalmology Clinics*, 1982; 22(3): 57–71.
6. Virgili G, Gatta G, Ciccolallo L, et al. Incidence of uveal melanoma in Europe. *Ophthalmology*, 2007; 114(12): 2309–2315.
7. Shields JS, Shields CL. Posterior uveal melanoma: clinical and pathologic features. In: Shields JA, Shields CL, editors. *Intraocular Tumours—A Text and Atlas*. Philadelphia Pa, USA: W.B. Saunders, 1992; 117–136.
8. Frenkel S, Hendler K, Pe'er J. Uveal melanoma in Israel in the last two decades: characterization, treatment and prognosis. *Israel Medical Association Journal*, 2009; 11(5): 280–285.
9. Seddon JM, Young TA. Epidemiology of uveal melanoma. In: Ryan SJ, editor. *The Retina*. 4th edition. Elsevier-Mosby, 2006; 691–698.
10. Schmidt-Pokrzywniak A, Jöckel KH, Bornfeld N, Sauerwein W, Stang A. Positive interaction between light iris color and ultraviolet radiation in relation to the risk of uveal melanoma: a case-control study. *Ophthalmology*, 2009; 116(2): 340–348.
11. Singh AD, Rennie IG, Seregard S, Giblin M, McKenzie J. Sunlight exposure and pathogenesis of uveal melanoma. *Survey of Ophthalmology*, 2004; 49(4): 419–428.
12. Landreville S, Agapova OA, Hartbour JW. Emerging insights into the molecular pathogenesis of uveal melanoma. *Future Oncology*, 2008; 4(5): 629–636.
13. Onken MD, Worley LA, Long MD, et al. Oncogenic mutations in GNAQ occur early in uveal melanoma. *Investigative Ophthalmology and Visual Science*, 2008; 49(12): 5230–5234.
14. Van Raamsdonk CD, Bezrookove V, Green G, et al. Frequent somatic mutations of GNAQ in uveal melanoma and blue naevi. *Nature*, 2009; 457(7229): 599–602.
15. Van Raamsdonk CD, Griewank KG, Crosby MB, et al. Mutations in GNA11 in uveal melanoma. *The New England Journal of Medicine*, 2010; 363(23): 2191–2199.
16. Brantley MA, Jr., Harbour JW. Deregulation of the Rb and p53 pathways in uveal melanoma. *American Journal of Pathology*, 2000; 157(6): 1795–1801.
17. Sun Y, Tran BN, Worley LA, Delston RB, Harbour JW. Functional analysis of the p53 pathway in response to ionizing radiation in uveal melanoma. *Investigative Ophthalmology and Visual Science*, 2005; 46(5): 1561–1564.
18. Ehlers JP, Worley L, Onken MD, Harbour JW. Integrative genomic analysis of aneuploidy in uveal melanoma. *Clinical Cancer Research*, 2008; 14(1): 115–122.
19. Shields JS, Shields CL. *Intraocular Tumours—An Atlas and Textbook 2008*. LWW; 2008. Iris melanoma, 22.
20. Laver NV, McLaughlin ME, Duker JS. Ocular melanoma. *Archives of Pathology and Laboratory Medicine*, 2010; 134(12): 1778–1784.
21. Shields CL, Furuta M, Berman EL, et al. Choroidal nevus transformation into melanoma: analysis of 2514 consecutive cases. *Archives of Ophthalmology*, 2009; 127(8): 981–987.
22. Shields CL, Shields JA, Kiratli H, De Potter P, Cater JR, McLean IW. Risk factors for growth and metastasis of small choroidal melanocytic lesions. *Ophthalmology*, 1995; 102(9): 1351–1361.
23. Hawkins BS. Schachat AP collaborative ocular melanoma study. In: Ryan SJ, editor. *The Retina*. 4th edition. Elsevier-Mosby, 2006; 803–810.

24. Seregard S, Damato B, Fleming P. Uveal malignant melanoma: management options—brachytherapy. In: Saunders A, editor. *Clinical Ophthalmic Oncology*, 2007; 241–247.
25. Mashayekhi A, Tuncer S, Shields CL, Shields JA. Tumour-lipid exudation after plaque radiotherapy of choroidal melanoma: the role of Bruch's membrane rupture. *Ophthalmology*, 2010; 117(5): 1013–1023.