Use of Formulated Thymoquinone as a Novel Adjuvant Antineoplastic for the Treatment of Follicular Lymphoma

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Introduction

Follicular lymphoma is the second most common type of Non-Hodgkin's lymphoma in the United States. As many patients are diagnosed late in the disease process, with widespread involvement of multiple lymph nodes and organ systems, ongoing research is focused on finding drugs with fewer side effects and minimal long-term risks. Thymoquinone, a natural compound derived from *Nigella sativa* (fennel flower), has shown strong apoptotic effects against lymphoma models both in culture and in vivo. However, thymoquinone's poor solubility and low bioavailability hinder its use as a chemotherapeutic agent. In this study, we aimed to overcome this challenge by using a novel nano-encapsulation technique to enhance the bioavailability and overall efficacy of the drug. We determined the IC50 values of nano-encapsulated thymoquinone, standard chemotherapeutic agents, and unencapsulated thymoquinone in WSU-FSCCL lymphoma cell lines to compare the efficacy of nano-encapsulation and characterize the potency of standard care chemotherapeutics. This work lays the foundation for future studies that will investigate combination killing effects of formulated thymoquinone alongside chemotherapeutics.

Results

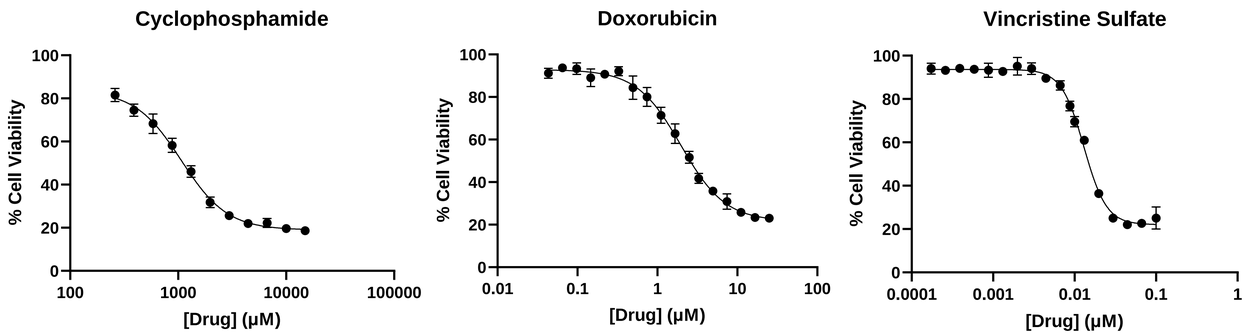


Figure 1: Standard of care chemotherapy cell viability data dose-response curves of Cyclophosphamide, Doxorubicin, and Vincristine Sulfate against FSCCL cell line, showcasing IC50 values of 1068 μM, 1.973 μM, and 0.01303 μM respectively

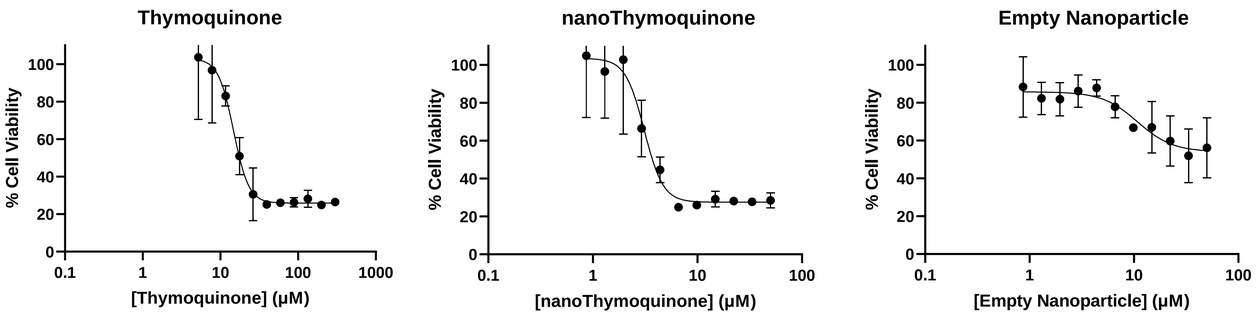
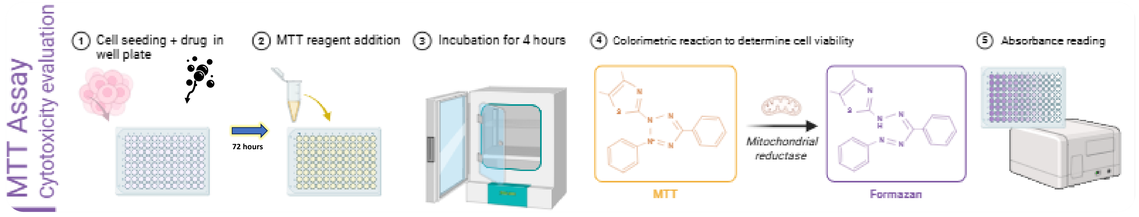


Figure 2: Standard of care chemotherapy cell viability data dose-response curves of Thymoquinone, nanoThymoquinone, and empty nanoparticle against FSCCL cell line, showcasing IC50 values of 14.78 μM, 3.101 μM, and N/A respectively

Methods

WSU-FSCCL cells were cultured in RPMI 1640 media with 10% FBS and 1% pen/strep. A minimum of four passages was performed to ensure maximal viability of cells. Cancer cells (10,000/well) were then seeded in four replicates per arm with 50 μL of complete media. 12-point 1:1.5 serial dilutions were performed for the cyclophosphamide, doxorubicin, and vincristine sulfate arms, and 50 μL of diluted drug was transferred to achieve a total working volume of 100 μL. Four replicates were carried out for the thymoquinone, nanothymoquinone, and vehicle arms, with dilutions consisting of 12-point 1:1.5 serial dilutions. Each experimental group included cell-only control wells. The plates were incubated at 37°C. Cellular proliferation and viability were assessed 72 hours later by adding 10 μL of 12 mM MTT per well and incubating for 4 hours at 37°C, followed by the addition of 100 μL of 0.1 M HCL and 0.35 M SDS, which were then incubated for another 4 hours at 37°C, before being read using a SpectraMax plate reader at 570nm.



Conclusions and Next Steps

Given the long-term disease course for patients with follicular lymphoma, the exploration of therapeutics that have fewer side effects and can be used safely over the long term has been on the rise. This experiment demonstrates that formulated thymoquinone exhibits approximately five times greater cytotoxic potency on FSCCL lymphoma cells compared to pure thymoquinone, highlighting its potential as a promising adjuvant therapy in combination with standard-of-care treatments. Additionally, these findings fully characterize the potency of three commonly administered chemotherapeutics on the WSU-FSCCL cell line, paving the way for future combination studies that demonstrate the adjuvant benefits of the formulated thymoquinone.

IC50 (μM)	
Cyclophosphamide	1068
Doxorubicin	1.973
Vincristine Sulfate	0.01303
Thymoquinone	14.78
nanoThymoquinone	3.101
Empty nanoParticle	N/A

Table 1: Standard of care chemotherapy and Thymoquinone variant cell viability calculated IC50s

Acknowledgement

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References

Schneider-Stock, R., et al., *Thymoquinone: fifty years of success in thebattle against cancer models*. Drug Discov Today, 2014. **19**(1): p. 18-30.

Methods Diagram (MTT Assay) created using BioRender. Access date 3/6/2025.