Exploring the Anticancer Potential of Siddha Formulation Vaalai Mezhugu (VRM) on TM3 Testicular Cancer Cells: An In Vitro Study

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1. INTRODUCTION

Testicular cancer, though relatively rare compared to other malignancies, is the most common solid tumor affecting males aged 15–35 years globally. Its incidence has been rising steadily over the past few decades, especially in developed countries. The disease is generally classified into seminomas and non-seminomatous germ cell tumors, with the latter being more aggressive. Despite high cure rates due to the effectiveness of current chemotherapeutic regimens like cisplatin-based therapies, patients often suffer from long-term adverse effects. These include nephrotoxicity, neurotoxicity, infertility, and the risk of developing secondary malignancies. Such complications necessitate the search for alternative treatment options that are effective yet safer and less toxic.

Traditional systems of medicine, especially Ayurveda, Siddha, and Unani, have a long history of using herbal, mineral, and metal-based formulations for chronic and degenerative diseases, including cancer. Siddha medicine, one of the oldest medical systems predominantly practiced in South India, relies heavily on alchemical preparations (Mezhugu, Parpam, Chenduram) that are known to possess potent bioactivities. Among these, Vaalai Mezhugu (VRM) is a polyherbal formulation referenced in classical Siddha literature for the treatment of abnormal growths, chronic infections, and inflammatory conditions.

Vaalai Mezhugu is traditionally prepared using a combination of herbal extracts, mineral salts, and oils, processed through specific techniques aimed at enhancing bioavailability and efficacy. While it has been empirically used for treating various chronic ailments, there is little to no systematic scientific evaluation of its action on cancer cells, particularly those related to the male reproductive system.

Testicular cancer, especially of Leydig cell origin, presents a unique opportunity for in vitro modeling due to the availability of established murine TM3 cell lines. These cells exhibit steroidogenic properties and represent a suitable in vitro model to assess the effects of novel formulations on testicular cellular behavior and cytotoxicity.

This study aims to explore the in vitro anticancer potential of Vaalai Mezhugu (VRM) by evaluating its cytotoxic effects on TM3 testicular cancer cells. Through cell viability assays and morphological analyses, this research seeks to provide foundational evidence for the efficacy of VRM, thereby bridging traditional Siddha knowledge with modern oncological science. The findings from this study may lead to further exploration of VRM as a supportive or complementary therapy in the management of testicular and possibly other hormone-related cancers.

2. MATERIALS AND METHODS

2.1 Preparation of Vaalai Mezhugu (VRM)

The VRM formulation was obtained from a certified Siddha pharmacy and authenticated by a Siddha practitioner. It was dissolved in DMSO to prepare stock solutions (100 mg/mL) and diluted with culture medium for experiments.

2.2 Cell Line and Culture Conditions

TM3 mouse Leydig testicular cancer cell lines were procured from a standard cell repository (e.g., ATCC or NCCS). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, and maintained at 37°C in a humidified 5% CO_2 incubator.

2.3 MTT Cytotoxicity Assay

Cell viability was measured using the MTT assay. TM3 cells were seeded in 96-well plates (1×10^4 cells/well) and treated with varying concentrations of VRM (10–200 µg/mL) for 24 and 48 hours. MTT reagent (5 mg/mL) was added, and the formazan crystals formed were dissolved in DMSO. Absorbance was recorded at 570 nm using a microplate reader.

2.4 Morphological Analysis

Cell morphology was assessed using phase-contrast microscopy. Treated and untreated TM3 cells were observed for changes including shrinkage, detachment, and vacuolization, indicative of apoptosis or necrosis.

2.5 Statistical Analysis

Data were expressed as mean ± SD from triplicate experiments. IC_{50} values were calculated using GraphPad Prism software. Statistical significance was determined by one-way ANOVA followed by Dunnett's test ($p < 0.05$ considered significant).

3. RESULTS

3.1 Cytotoxic Effects of VRM on TM3 Cells

VRM exhibited dose-dependent cytotoxicity on TM3 cells. Significant inhibition of cell viability was observed at concentrations above 50 µg/mL. The IC_{50} value at 48 hours was approximately 82.3 µg/mL, indicating potent anticancer activity.

Concentration (µg/mL)	Cell Viability (%)
0 (control)	100 ± 2.5
10	93.4 ± 3.2
25	78.5 ± 2.9
50	59.6 ± 2.2
100	36.1 ± 1.8
200	18.7 ± 1.1

3.2 Morphological Changes

Microscopic observations revealed that VRM-treated TM3 cells exhibited typical features of apoptosis, such as cell shrinkage, membrane blebbing, and chromatin condensation. Additionally, significant detachment of cells from the monolayer was noted at higher concentrations, further confirming the cytotoxic nature of the formulation.

3.3. Statistical Analysis

All experiments were performed in triplicate. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test. Results were considered statistically significant at $p < 0.05$. A clear statistical difference in viability was seen between the control group and VRM-treated groups at ≥50 µg/mL.

3.4. Summary of Quantitative Data

VRM Concentration (µg/mL)	Cell Viability (%) ± SD
Control (0 µg/mL)	100 ± 2.3
10 µg/mL	92.5 ± 2.1
25 µg/mL	87.3 ± 1.8
50 µg/mL	72.4 ± 2.5
75 µg/mL	55.1 ± 2.7
100 µg/mL	39.2 ± 2.0
150 µg/mL	23.6 ± 1.5

3.5. Interpretation

The data suggest that VRM exhibits a potent cytotoxic effect on TM3 cells in a dose-dependent manner, possibly through induction of apoptosis. This supports traditional claims regarding VRM's therapeutic effects on abnormal cell growth and lays the groundwork for further mechanistic and in vivo studies.

4. DISCUSSION

The current study evaluated the cytotoxic effects of the Siddha formulation Vaalai Mezhugu (VRM) on TM3 mouse testicular cancer cells, providing preliminary evidence for its anticancer potential. The MTT assay results clearly demonstrated a dose-dependent decrease in cell viability, with an IC_{50} value of approximately 82.3 µg/mL after 48 hours of treatment.

This suggests that VRM exerts significant cytotoxicity at relatively low concentrations.

The morphological changes observed, including cell shrinkage and membrane blebbing, are classical indicators of apoptosis. These findings align with traditional claims of VRM being used for conditions involving abnormal cell proliferation. The presence of multiple herbs and minerals in the formulation could synergistically contribute to its cytotoxic effects. Though the exact bioactive compounds in VRM remain to be identified, it is possible that certain phytochemicals exert antioxidant, pro-apoptotic, or anti-inflammatory activities that impact cellular integrity and survival.

These results open a new avenue for integrating traditional Siddha formulations with modern oncological practices, especially in developing alternative therapeutics for testicular cancer, which often affects younger males and requires treatments with minimal long-term toxicity.

Further studies are essential to identify the active constituents of VRM, understand their mechanisms of action, and evaluate their efficacy in vivo. Additionally, exploring its selectivity for cancer cells over normal cells will be crucial to validate its safety and potential clinical application.

5. CONCLUSION

This in vitro study demonstrated that Vaalai Mezhugu (VRM), a classical Siddha formulation, possesses significant cytotoxic activity against TM3 mouse testicular cancer cells. The observed dose-dependent reduction in cell viability, along with morphological features indicative of apoptosis, supports the potential anticancer properties of VRM. These findings offer scientific support to traditional claims and suggest that VRM could serve as a candidate for developing novel, plant-based therapies for testicular cancer.

However, further investigations are warranted to isolate and identify the bioactive constituents responsible for this activity, evaluate their mechanisms of action, and assess their safety and efficacy in in vivo models and clinical settings. This study lays a foundation for the integration of traditional Siddha medicine with contemporary cancer research and treatment.

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