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IN VITRO CYTOTOXICITY STUDY OF KOWSIKAR KULAMBU- A SIDDHA FORMULATION

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ABSTRACT
Kowsikar kulambu is a classical siddha formulation indicated for various disease conditions. Scientific validation of its safety is essential for its therapeutic application. The present study aimed to evaluate the cytotoxic potential of kowsikar kulambu using (MTT assay). The formulation of the medicine was present in siddha vaidhiya thirattu, a classical literature and the medicine was subjected to cytotoxicity analysis at different concentrations. Result demonstrated a dose dependent response, with mention IC50 value. The findings suggest that kowsikar kulambu is non toxic at therapeutic concentrations, thereby supporting its safe use in clinical practice.

KEYWORDS: Kowsikar kulambu, Cytotoxicity, Siddha formulation, MTT assay.

BACKGROUND
Kowsikar kulambu is a classical siddha herbomineral formulation mentioned in traditional siddha text. It has been used in clinical practice for management of certain diseases like anorectal disease, skin disease, vadha disease. With the global interest in complementary and alternate medicine, it is essential to scientifically validate the properties of the medicine. In this context, invitro cytotoxicity assays serve as a primary step in drug development to determine whether a formulation possesses toxic effects on normal or diseased cells. MTT assay is a colorimetric assay used for the determination of cell proliferation and cytotoxicity, based on reduction of the yellow colored water soluble tetrazolium dye MTT to formazan crystals. Mitochondrial lactate dehydrogenase produced by live cells reduces MTT to insoluble formazan crystals, which upon dissolution into an appropriate solvent exhibits purple colour, the intensity of which is proportional to the number of viable cells and can be measured spectrophotometrically at 570nm. (Alley et al and Mosamann et al)

AIM AND OBJECTIVE
The study aimed to evaluate the cytotoxic potential of kowsikar kulambu using MTT assay in Human dermal fibroblast cells.

MATERIALS AND METHODS
Materials and methods section of this study relies on a comprehensive review of both siddha and modern literature and relevant articles.

1. Cell lines: HDF-Human dermal fibroblasts
2. DMEM with high glucose medium
3. Fetal Bovine Serum

PROCEDURE
MAINTENANCE OF CELL LINES
The HDF (Human dermal fibroblast cell line) was purchased from HiMedia laboratories, Mumbai, India. The cells were maintained in DMEM-high glucose media supplemented with 10% FBS along with the 1% antibiotic-antimycotic solution in the atmosphere of 5% CO2, 18-20% O2 at 370C temperature in the CO2 incubator and sub-cultured for every 2days. Passage of number of HDF cells was 18 used for the current study.

STEPS FOLLOWED
1. Seed 200µl cell suspension in a 96-well plate at the required cell density (15,000 cells per well) and allow the cells to grow for about 24 hours. 2. Add appropriate concentrations of the given test compound diluted in culture media(Mentioned in the results - Excel sheet) 3. Incubate the plate for 24hrs at 37°C in a 5% CO2 atmosphere. 4. After the incubation period, takeout the plates from incubator and remove spent media and add

MTT reagent to a final concentration of 0.5mg/ml of total volume. 5. Wrap the plate with aluminium foil to avoid exposure to light. 6. Return the plates to the incubator and incubate for 3 hours. (Note: Incubation time varies for different cell lines. Within one experiment, incubation time should be kept constant while making comparisons.) 7. Remove the MTT reagent and then add 100µl of solubilisation solution (DMSO). 8. Gentle stirring in a gyratory shaker will enhance dissolution. Occasionally, pipetting up and down may be required to completely dissolve the MTT formazan crystals especially in dense cultures. 9. Read the absorbance on a spectrophotometer or an ELISA reader at 570nm wavelength. 10. % Cell viability is calculated using below formula: % cell viability=[Mean abs of treated cells/Mean abs of Untreated cells] x 100. 11. The IC50 value was determined by using linear regression equation i.e. Y =Mx+C. Here, Y = 50, M and C values were derived from the viability graph.

TEST CONCENTRATIONS DETAILS

In this study, the given test compound was evaluated for its cytotoxic effects on HDF cells using the MTT assay. The cells were treated with varying concentrations of the test compound to assess dose-dependent cytotoxicity. The concentrations used for treatment were as follows:

Table 1: Details of drug treatment to respective cell lines used for the study.

SI.No	Culture condition	Cell lines	Concentration treated to cells
1	Untreated	HDF	No treatment
2	Blank	-	Only media without cells
3	KK	HDF	5(6.25,12.5,25,100 ug/ml)
4	Doxorubicin	HDF	1ug/ml

DISCUSSION

In this study, the medicine at lower concentration of 6.25ug it achieves 99.77% cell viability and the highier concentration of 100ug it achieves 53.28% cell viability and the standard comparative medicine doxorubicin achieves 53.28. Our findings indicate that kowsikar kulambu exhibited a dose dependent cytotoxic effect, the formulation maintained cell viability within acceptable limits, suggesting its safety at therapeutic doses.

RESULTS

Table 2: Percentage cell viability of HDF cells treated with different concentrations of KK after 24 hours, along with the observed IC_{50} value.

Cell viability data-kowsikar kulambu(kk)		
Culture condition	**%cell viability**	**IC50 conc(ug/ml)**
Untreated	100.00	**109.88**
Toxin – 1ug	51.90	
kk-6.25ug	99.77	
kk-12.5ug	88.73	
kk-25ug	78.23	
kk-50ug	61.75	
kk-100ug	53.28	

CONCLUSION

The present in vitro cytotoxicity study of kowsikar kulambu, a classical siddha formulation, demonstrated that the medicine possesses promising cytotoxic potential in a dose-dependent manner. The findings suggest that the formulation contains bioactive constituents capable of exerting inhibitory effects on cell viability, supporting its traditional claims of therapeutic efficacy. However, to fully understand its molecular mechanism of action, additional in vitro studies are necessary.

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