Evaluation of cytotoxic effects of Siddha formulation Thamira Kattu Chendooram versus standard CHOP chemotherapeutic agents against hematological malignancy cell lines

R. Madheswaran[1]*, P. Srinivasan[2], K. Sukumar[3], M. Arthanari Eswaran[4]

[1]Department of Veterinary Pathology, [2]Poultry Disease Diagnosis and Surveillance Laboratory, [3]Department of Veterinary Microbiology, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Namakkal - 637 002, Tamil Nadu, India; [4]Department of Veterinary Microbiology, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Udumalpet - 642 205, Tamil Nadu, India.
*Corresponding Author E-mail: rmadheswaran2003@gmail.com

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Abstract

A study was conducted to evaluate the comparative *in vitro* anticancer activity of the Siddha formulation Thamira Kattu Chendooram (TKC) and standard CHOP therapy (cyclophosphamide, doxorubicin, vincristine and prednisone) against HL-60 and Jurkat cell lines using MTT cytotoxicity assay. The cells were exposed to varying concentrations of TKC and CHOP drugs for 48 hours and cytotoxicity was determined by IC_{50} values using nonlinear regression analysis. In HL-60 cells, the IC_{50} values of TKC and CHOP drugs were 16.55, 2.197, 1.334, 8.396 and 17.88 µM respectively. In Jurkat cells, the corresponding IC_{50} values were 17.84, 3.072, 1.489, 8.627 and 18.39 µM. Among these drugs, doxorubicin exhibited the highest cytotoxic potency, followed by cyclophosphamide and vincristine, whereas TKC showed moderate antiproliferative activity compared to prednisone in both cell lines. The concentration-dependent cytotoxic response observed with TKC suggests its potential anticancer activity, possibly mediated through the mechanisms involved in cell cycle arrest and apoptosis. These findings indicated that TKC possesses appreciable *in vitro* anticancer activity and may serve as a promising therapeutic candidate for hematological malignancies.

Keywords: MTT assay, HL-60 cells, Jurkat cells, CHOP therapy, TKC

Introduction

Lymphoproliferative disorders (LPDs) are a group of hematological malignancies that affect both humans and animals. It is associated with impaired immune function resulting from immunodeficiency and immunodysregulatory syndromes. Among LPDs, lymphoma is one of the most common neoplasms of hematopoietic tissues, arising from abnormal transformation and uncontrolled proliferation of lymphocytes. Lymphomas are broadly classified into Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL), accounting for approximately 10% and 90% of all lymphomas, respectively (Mugnaini and Ghosh, 2016). Globally, NHL ranges from a relatively rare disease to the fifth most common cancer, depending on geographical distribution (Nair et al., 2016). In India, the incidence of NHL has shown a steady increase, with urban cancer registries reporting 5.1 cases per 1,00,000 population (Nair et al., 2016). The hematological malignancies are the common tumours in dogs (Teske et al., 1994, Dash, 2025), in which NHL alone accounting for 80% of cases (Valli et al., 2013). Approximately 60 to 70% of lymphomas originate from B-lymphocytes and 30 to 40% arise from T-lymphocytes (Meuten, 2017; Swerdlow et al., 2017).

Chemotherapy remains the standard treatment for hematological malignancies; however, its clinical utility is often restricted by high cost, prolonged treatment duration, adverse effects and disease relapse. Multi-drug treatment protocols are widely employed, among them CHOP regimen comprising cyclophosphamide, hydroxydaunorubicin (doxorubicin), vincristine and prednisolone/prednisone is considered the gold standard (Lubgan et al., 2009; Pawlak et al., 2014). This protocol achieves 80-90% complete response rates, with a median survival of approximately 12 months. Although advances in targeted therapy continue to emerge, the identification of safer and more effective anticancer agents remains a major challenge. Thamira Kattu Chendooram (TKC) is a classical Siddha herbo-mineral formulation traditionally used for the treatment of various cancers (Vasudha Devi et al., 2014). Previous studies have demonstrated its cytotoxic activity against multidrug-resistant cancer cell lines (Arul Amuthan et al., 2013). However, despite its longstanding clinical use, systematic evaluation of its anticancer efficacy remains limited. Therefore, the present study was undertaken to evaluate the comparative *in vitro* anticancer activity of TKC and CHOP therapy against hematological malignancy cell lines.

Materials and Methods

Drugs

The Siddha medicine TKC (25 g) was procured from M/s SKM Siddha and Ayurvedha Company Ltd., Erode, India and stored at room temperature until further use. The standard anti-lymphoma drug cyclophosphamide (5 g) was purchased from M/s TCI Chemicals, Chennai and maintained under refrigerator (2-8°C). The anticancer drug doxorubicin (500 mg) was received as a complimentary sample from M/s Dr. Reddy's Laboratories Ltd., Hyderabad and stored at room temperature. The chemotherapeutic agent vincristine (1 mg) was procured from M/s Celon Laboratories Pvt. Ltd., Hyderabad and preserved under refrigerator (2-8°C). The corticosteroid prednisone (1 g) was purchased from M/s TCI Chemicals, Chennai and stored at room temperature.

Cell lines, Medium and Buffers

Human promyelocytic leukemia cell line HL-60 and T-lymphocyte cell line Jurkat were procured from the National Centre for Cell Sciences (NCCS), Pune, India and maintained at 37°C in a CO_2 incubator. The cell culture media, Iscove's Modified Eagle Medium (IMEM) and Roswell Park Memorial Institute (RPMI)-1640 medium, were procured from M/s HiMedia Laboratories, Mumbai and stored at 4°C until use. The dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from M/s Sigma-Aldrich, USA and stored under refrigeration (2-8°C). Dimethyl sulphoxide (DMSO) (100 mL), fetal bovine serum (FBS) (500 mL) and phosphate-buffered saline (PBS) 10x, pH 7.2 (500 mL) were procured from M/s HiMedia Laboratories, Mumbai, with DMSO and PBS stored at room temperature and FBS preserved in a deep freezer at −20°C.

***In vitro* cytotoxicity assay**

The cytotoxicity was determined separately for HL-60 and Jurkat cell lines using MTT assay as described by Ghasemi et al. (2021).

The HL-60 cells were subcultured in IMEM supplemented with 10% FBS and 1% antibiotic-antimycotic solution containing penicillin (100 IU/mL) and streptomycin (100 µg/mL). The cells were seeded into 96-well microplates and treated with different concentrations of TKC (0.10, 0.70, 3.00, 11.00, 45.00, 140.00, 280.00, 1120.00 µM), cyclophosphamide (0.01, 0.10, 0.40, 1.50, 6.00, 18.00, 38.00, 150.00 µM), doxorubicin (0.01, 0.05, 0.25, 1.00, 4.00, 12.50, 25.00, 100.00 µM), vincristine (0.05, 0.4, 1.50, 6.25, 25.00, 78.00, 156.00, 625.00 µM) and prednisone (0.10, 0.75, 3.00, 12.00, 48.00, 150.00, 300.00, and 1200.00 µM).

The Jurkat cells were subcultured in RPMI-1640 medium supplemented with 10% FBS and 1% antibiotic-antimycotic solution containing penicillin (100 IU/mL) and streptomycin (100 µg/mL). The cells were seeded into 96-well plates and treated with varying concentrations of TKC (0.10, 0.70, 3.25, 12.00, 47.50, 148.50, 297.00, 1188.00 µM), cyclophosphamide (0.02, 0.12, 0.50, 2.00, 8.00, 25.00, 50.00, 200.00 µM), doxorubicin (0.01, 0.06, 0.30, 1.20, 4.80, 15.00, 30.00, 120.00 µM), vincristine (0.05, 0.40, 1.60, 6.50, 26.00, 81.25, 162.50, 650.00 µM) and prednisone (0.10, 0.80, 3.40, 12.75, 51.00, 159.40, 318.80, 1275.00 µM).

All drugs were dissolved in DMSO, which was also added to the culture well as the vehicle control. The plates were incubated for 48 hour at 37°C in a humidified atmosphere containing 5% CO_2 to allow adequate cell growth and drug interaction. After incubation, the culture medium was removed and the cells were washed twice with PBS. Subsequently, 10 µL of MTT solution (5 mg/mL) was added to each well and incubated for 4 hours at 37°C in 5% CO_2. After incubation, absorbance was measured at 595 nm using iMax Microtitre Plate Reader (M/s Biorad Medisystems, Mumbai). The cytotoxicity (IC_{50}) values were calculated using GraphPad Prism 5.02 and data were fitted into sigmoidal dose-response nonlinear regression curve. The IC_{50} values were presented with 95% confidence intervals. All treatments were performed in triplicate to ensure the reproducibility of the results.

Results

Cytotoxicity of HL-60 cells

The HL-60 cells were exposed to varying concentrations of TKC and CHOP drugs. The inhibitory effect of the drugs on cell proliferation was found to be concentration-dependent, reflecting their cytotoxic potential (Fig. 1-5). TKC and prednisone exhibited moderate cytotoxic activity, while vincristine demonstrated marked cytotoxicity against HL-60 cells. Cyclophosphamide and doxorubicin produced severe cytotoxic effects, indicating their greater antiproliferative effects. The IC_{50} values were confirmed the cytotoxic response of HL-60 cells to TKC and CHOP treatments. The IC_{50} values for TKC and CHOP drugs were 16.55, 2.197, 1.334, 8.396 and 17.88 µM respectively (Fig. 6-10). Among these drugs, doxorubicin exhibited the highest cytotoxic potency with the lowest IC_{50} value, followed by cyclophosphamide and vincristine, whereas TKC exhibited moderate cytotoxic activity with IC_{50} values numerically similar to those observed for prednisone under the present experimental conditions against HL-60 cells.

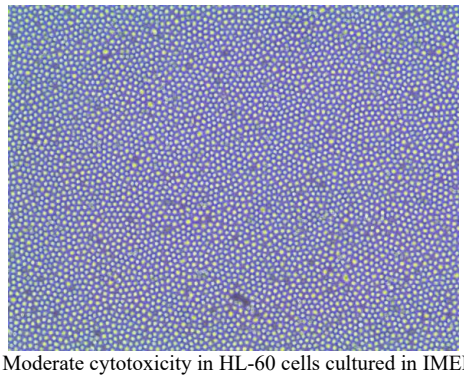


Fig. 1 Moderate cytotoxicity in HL-60 cells cultured in IMEM medium treated with TKC (200x)

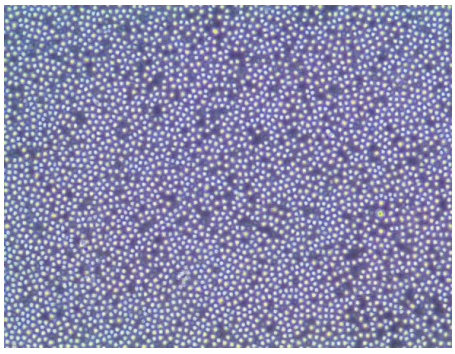


Fig. 2 Severe cytotoxicity in HL-60 cells cultured in IMEM medium treated with cyclophosphamide (200x)

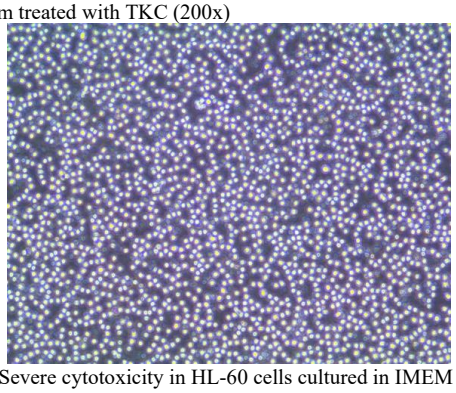


Fig. 3 Severe cytotoxicity in HL-60 cells cultured in IMEM medium treated with doxorubicin (200x)

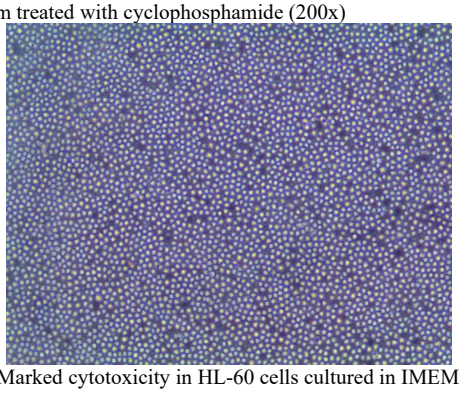


Fig. 4 Marked cytotoxicity in HL-60 cells cultured in IMEM medium treated with vincristine (200x)

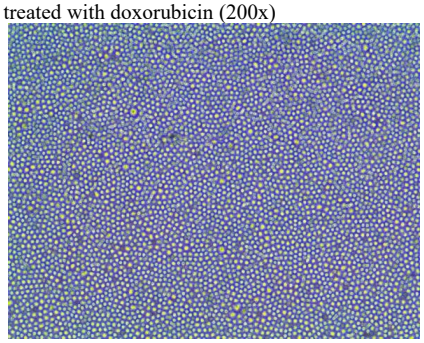


Fig. 5 Moderate cytotoxicity in HL-60 cells cultured in IMEM medium treated with prednisone (200x)

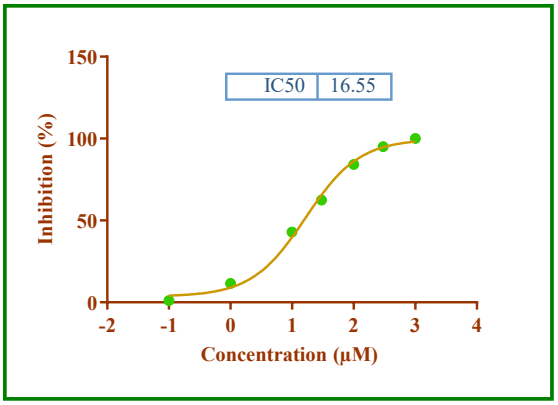


Fig. 6 Dose response curve and IC_{50} value of TKC on HL-60 cell line

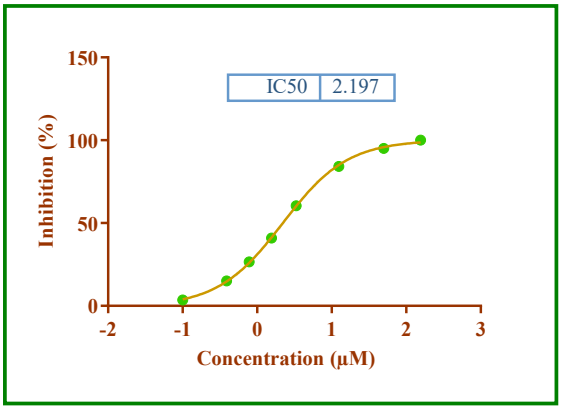


Fig. 7 Dose response curve and IC_{50} value of cyclophosphamide on HL-60 cell line

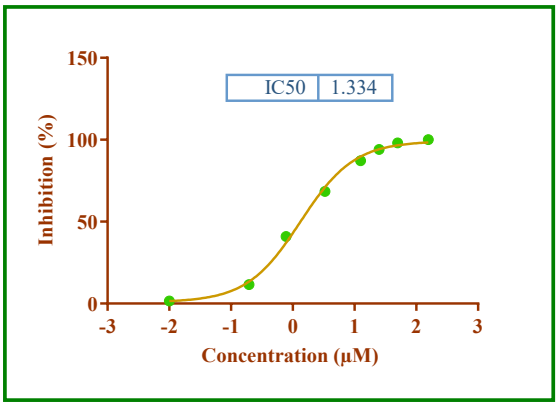


Fig. 8 Dose response curve and IC_{50} value of doxorubicin on HL-60 cell line

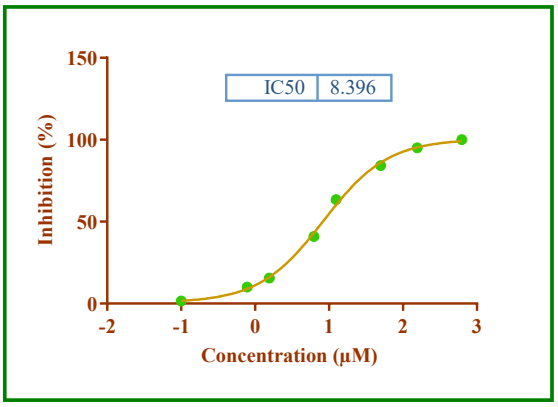


Fig. 9 Dose response curve and IC_{50} value of vincristine on HL-60 cell line

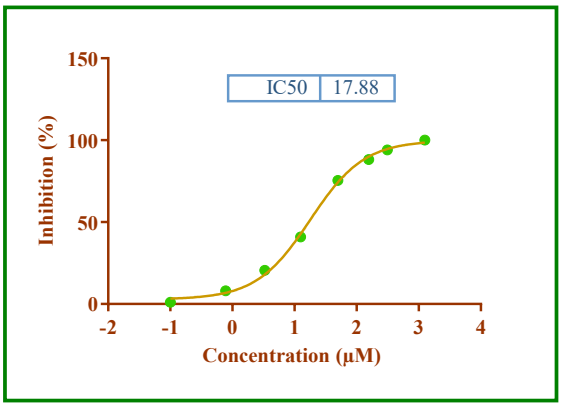


Fig. 10 Dose response curve and IC_{50} value of prednisone on HL-60 cell line

Cytotoxicity of Jurkat cells

The Jurkat cells were exposed to varying concentrations of TKC and CHOP drugs. The inhibitory effect of the drugs on cell proliferation was found to be concentration-dependent, reflecting their cytotoxic potential (Fig. 11-15). TKC and prednisone exhibited moderate cytotoxic activity, while vincristine showed marked cytotoxicity against Jurkat cells. Doxorubicin and cyclophosphamide produced severe cytotoxic effects, indicating their greater antiproliferative effects. The IC_{50} values were confirmed the cytotoxic response of Jurkat cells to TKC and CHOP treatments. The IC_{50} values for TKC and CHOP drugs were 17.84, 3.072, 1.489, 8.627 and 18.39 µM respectively (Fig. 16-20). Among these drugs, doxorubicin exhibited the highest cytotoxic potency with the lowest IC_{50} value, followed by cyclophosphamide and vincristine, whereas TKC exhibited moderate cytotoxic activity with IC_{50} values numerically similar to those observed for prednisone under the present experimental conditions against Jurkat cells.

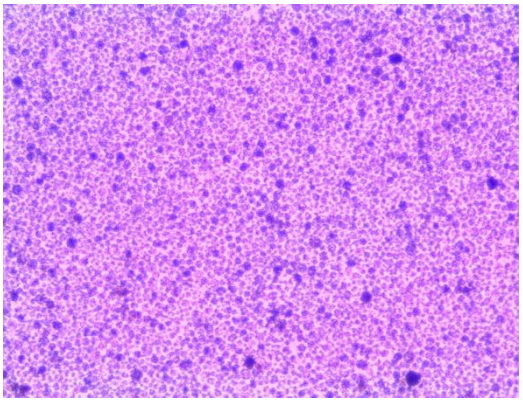


Fig. 11 Moderate cytotoxicity in Jurkat cells cultured in RPMI-1640 medium treated with TKC (200x)

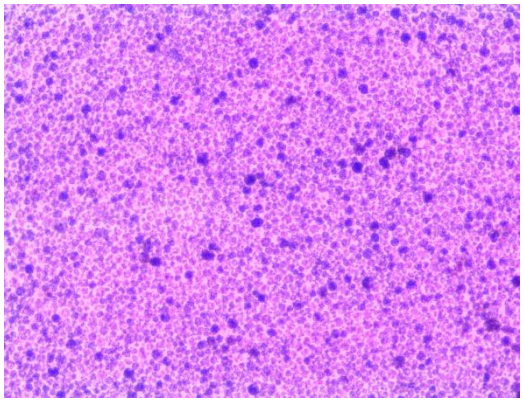


Fig. 12 Severe cytotoxicity in Jurkat cells cultured in RPMI-1640 medium treated with cyclophosphamide (200x)

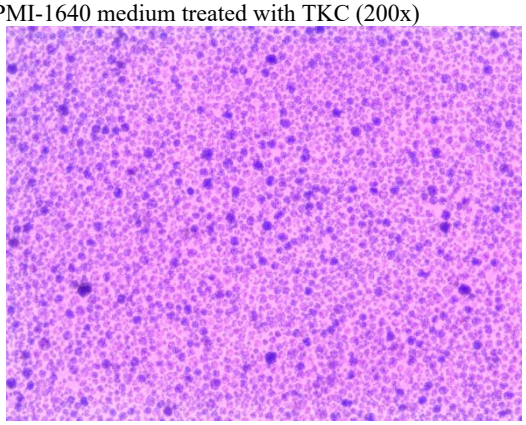


Fig. 13 Severe cytotoxicity in Jurkat cells cultured in RPMI-1640 medium treated with doxorubicin (200x)

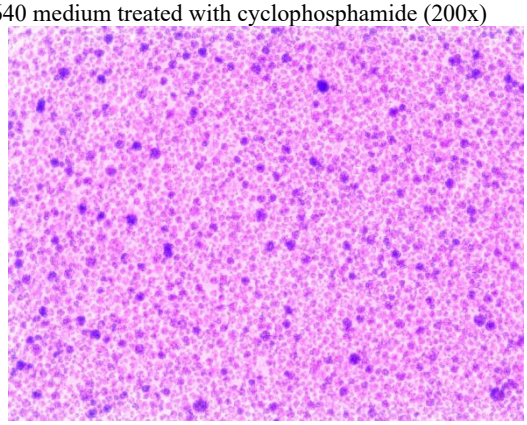


Fig. 14 Marked cytotoxicity in Jurkat cells cultured in RPMI-1640 medium treated with vincristine (200x)

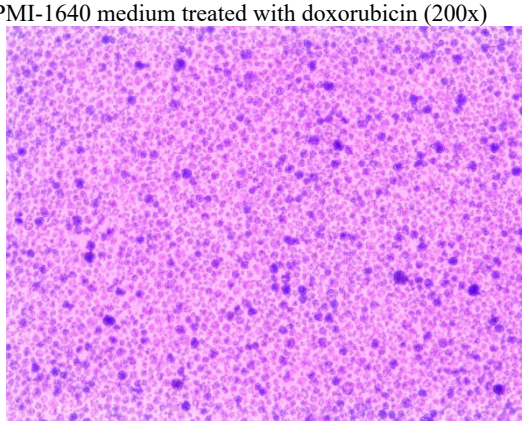


Fig. 15 Moderate cytotoxicity in Jurkat cells cultured in RPMI-1640 medium treated with prednisone (200x)

Discussion

The present study demonstrated the IC_{50} values of TKC and CHOP drugs against HL-60 cells indicated variable antiproliferative efficacy. Among the CHOP drugs, doxorubicin exhibited the highest cytotoxic potency, followed by cyclophosphamide and vincristine, while prednisone showed comparatively lower activity. The IC_{50} value of cyclophosphamide observed in the present study was markedly lower in HL-60S and HL-60R cell lines, suggesting enhanced sensitivity of the HL-60 cells used in this study. Cyclophosphamide requires metabolic activation *in vivo*, and therefore its cytotoxic activity observed in cell culture may not fully reflect its clinical pharmacological effects. The IC_{50} value of doxorubicin was consistent with the findings of Lubgan et al. (2009) confirming its established efficacy against leukemic cells. The TKC exhibited moderate cytotoxic activity with an IC_{50} value comparable to prednisone, indicating its potential antiproliferative effect against HL-60 cells. Although comparative literature on TKC and prednisone against HL-60 cell lines remains limited, the present findings suggest that TKC possesses measurable anticancer potential and warrants further mechanistic and *in vivo* investigations to validate its therapeutic relevance.

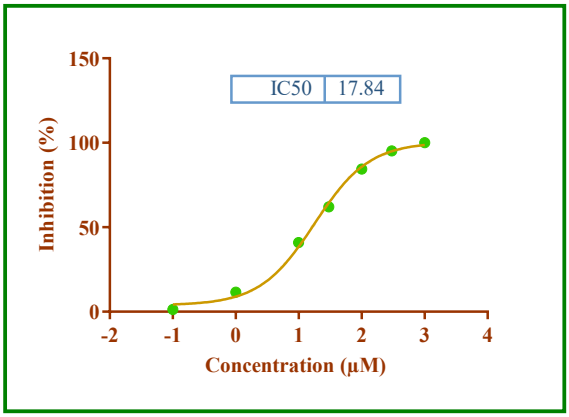


Fig. 16 Dose response curve and IC_{50} value of TKC on Jurkat cell line

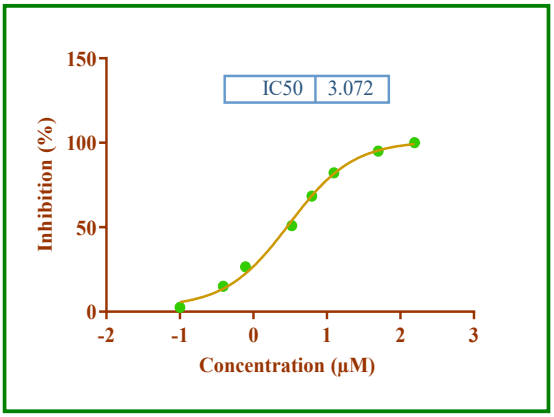


Fig. 17 Dose response curve and IC_{50} value of cyclophosphamide on Jurkat cell line

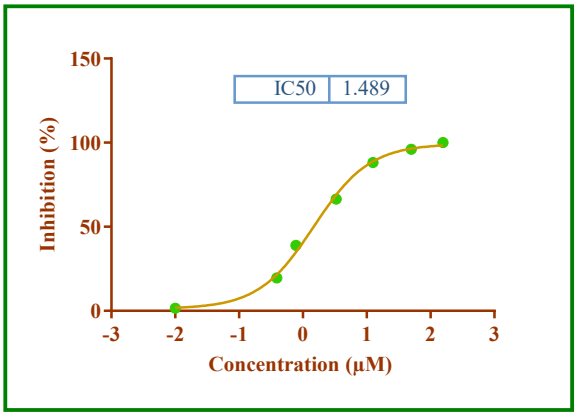


Fig. 18 Dose response curve and IC_{50} value of doxorubicin on Jurkat cell line

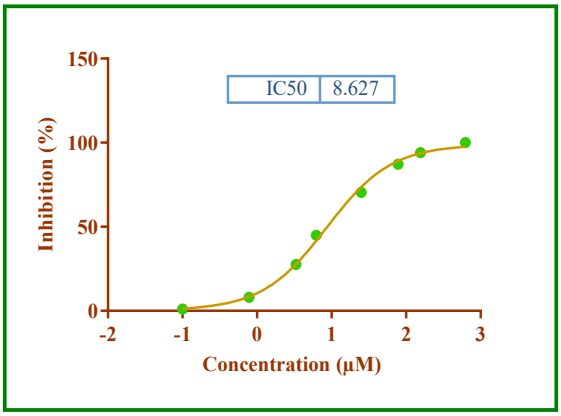


Fig. 19 Dose response curve and IC_{50} value of vincristine on Jurkat cell line

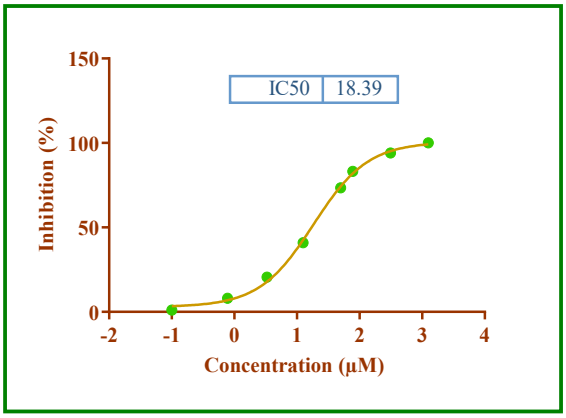


Fig. 20 Dose response curve and IC_{50} value of prednisone on Jurkat cell line

The IC_{50} values of TKC and CHOP drugs against Jurkat cells indicate differential cytotoxic responses. Among the CHOP drugs, doxorubicin exhibited the highest cytotoxic potency, followed by cyclophosphamide and vincristine, whereas prednisone showed comparatively lower antiproliferative activity. These findings were in agreement with the observations of Pawlak et al. (2014) who reported comparable IC_{50} values for CHOP drugs in Jurkat cells. The strong cytotoxic effect of doxorubicin and cyclophosphamide confirms their established therapeutic role in hematological malignancy therapy, while vincristine exhibited moderate but significant antiproliferative effects. The TKC demonstrated moderate cytotoxic activity with an IC_{50} value comparable to prednisone, suggesting its potential inhibitory effect on Jurkat cell proliferation. Although literature regarding the cytotoxic evaluation of TKC against Jurkat cells is currently unavailable, the comparable efficacy observed with standard CHOP drugs highlights its possible therapeutic relevance.

The antiproliferative and cytotoxic effects of TKC and CHOP drugs against HL-60 and Jurkat cells attributed to their ability to induce cell cycle arrest and trigger apoptotic pathways, ultimately leading to inhibit

leukemic cell proliferation. Previous studies have demonstrated that CHOP drugs exert their cytotoxic effects through disruption of DNA replication, microtubule destabilization, oxidative stress and activation of intrinsic apoptotic signaling pathways in hematopoietic cells, resulting in programmed cell death and growth suppression (Lubgan et al., 2009; Pawlak et al., 2014). The comparable cytotoxic response exhibited by TKC in both HL-60 and Jurkat cells suggests that its antiproliferative activity may involve similar molecular mechanisms, possibly through modulation of cell cycle regulatory checkpoints and apoptotic cascades.

Conclusion

The TKC exhibited moderate *in vitro* anticancer activity against HL-60 and Jurkat cells, with cytotoxic efficacy comparable to prednisone, among the standard drugs used in CHOP therapy. The underlying mechanisms responsible for the observed cytotoxic effects were not investigated in the present study and warrant further evaluation using apoptosis and cell-cycle assays. These findings highlight the therapeutic potential of TKC as a candidate for the development of anticancer formulations. Further molecular and *in vivo* studies are required to elucidate its precise mechanisms of action and validate its clinical applicability in the treatment of hematological malignancies.

Author's contribution: Dr. R. Madheswaran, performed the study and prepared the draft manuscript, Dr. P. Srinivasan, reviewed the drafted manuscript, Dr. K. Sukumar, interpretation of the data and Dr. M. Arthanari Eswaran, technical assistance in the study.

Conflict of interest: The authors declare that no conflict of interest of any type for this work.

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