Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 22s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Exploring The Anti-Cancer Potential of Triphala: A Study on Leukemia Cell Lines

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Cite this paper as: Shivraj Vilas Mane, Ravindra Pal Singh, (2025). Exploring The Anti-Cancer Potential of Triphala: A Study on Leukemia Cell Lines. *Journal of Neonatal Surgery*, 14 (22s), 420-425.

ABSTRACT

Cancer, particularly blood cancers like leukemia, represents a significant public health concern worldwide, with limited treatment options and severe side effects associated with conventional therapies. Traditional medicine, including Ayurvedic formulations, offers a promising alternative for managing these conditions with fewer adverse effects. Triphala, a polyherbal formulation consisting of Emblica officinalis, Terminalia chebula, and Terminalia bellirica, is known for its antioxidant, anti-inflammatory, and immunomodulatory properties. Recent studies suggest that Triphala may exert anti-cancer effects through apoptosis induction, angiogenesis inhibition, and modulation of cancer cell survival pathways. However, its specific effects on blood cancer cell lines have not been extensively studied. The aim of this study was to evaluate the cytotoxic effects of Triphala on human blood cancer cell lines HL-60 (human promyelocytic leukemia) and K562 (chronic myelogenous leukemia). Aqueous of Triphala were prepared and used to treat the cells at concentrations ranging from 25 to 200 µg/ml for 24, 48, and 72 hours. The cytotoxicity was assessed using the MTT assay, and cell viability was measured at 570 nm. The results demonstrated that Triphala exhibited dose- and time-dependent cytotoxicity in both cell lines, with HL-60 cells being more sensitive than K562 cells. The IC_{50} values for HL-60 and K562 cells were approximately 90 µg/ml and 110 µg/ml, respectively. These findings suggest that Triphala holds significant promise as a therapeutic agent for blood cancers, warranting further investigation into its mechanisms of action, molecular targets, and potential clinical applications.

Keyword: *Anti-cancer, Cytotoxicity, HL-60, K562, Triphala*

INTRODUCTION

Cancer is one of the leading causes of morbidity and mortality worldwide, with blood cancers, including leukemia, lymphoma, and myeloma, representing a significant burden on public health. Despite advancements in conventional treatments such as chemotherapy, radiotherapy, and bone marrow transplantation, these methods are often associated with severe side effects and limited long-term efficacy. Hence, there is an increasing interest in exploring alternative and complementary therapies that offer less toxicity and improved outcomes. One such promising approach involves the use of traditional medicinal formulations derived from natural products. Among these, Triphala, a polyherbal Ayurvedic formulation, has garnered significant attention for its potential therapeutic effects in various malignancies.[iiiiii]

Triphala is a blend of three medicinal fruits: Emblica officinalis (Amla), Terminalia chebula (Haritaki), and Terminalia bellirica (Bibhitaki). It is well-documented for its antioxidant, anti-inflammatory, and immunomodulatory properties. Recent preclinical studies suggest that Triphala exerts anti-cancer effects by inducing apoptosis, inhibiting angiogenesis, and modulating signaling pathways critical to cancer cell survival. While much research has focused on its activity in solid tumors, its specific effects on blood cancer cell lines remain underexplored. Investigating its bioactive components and their mechanisms of action in hematological malignancies could provide valuable insights into developing novel, plant-based therapeutic strategies.[iv]

The present study aims to investigate the anti-cancer potential of Triphala in blood cancer cell lines. By elucidating these mechanisms, this study seeks to contribute to the growing body of evidence supporting the use of traditional herbal formulations in cancer therapy and to explore Triphala's potential role as an adjunctive or alternative treatment for blood cancers.[v]

MATERIALS AND METHODS[viviiviiiixx]

1. Cell Lines Used

The study was conducted using two human blood cancer cell lines.

- HL-60 represents a human promyelocytic leukemia cell line.
- K562 represents a human chronic myelogenous leukemia cell line.

2. Preparation for Triphala Extract

- Triphala tablet was prepared.
- Aq. extracts were prepared: using Soxhlet extraction methods.

3. Cytotoxicity Assay

- The cytotoxicity of Triphala was assessed using the MTT assay to determine cell viability.
- Cells (1×10^4 per well) were seeded in 96-well plates and treated with Triphala at concentrations of 25, 50, 100, and 200 µg/ml for 24, 48, and 72 hours.
- After the treatment period, MTT reagent was added to each well, followed by incubation.
- Absorbance was measured at 570 nm to evaluate cell viability.

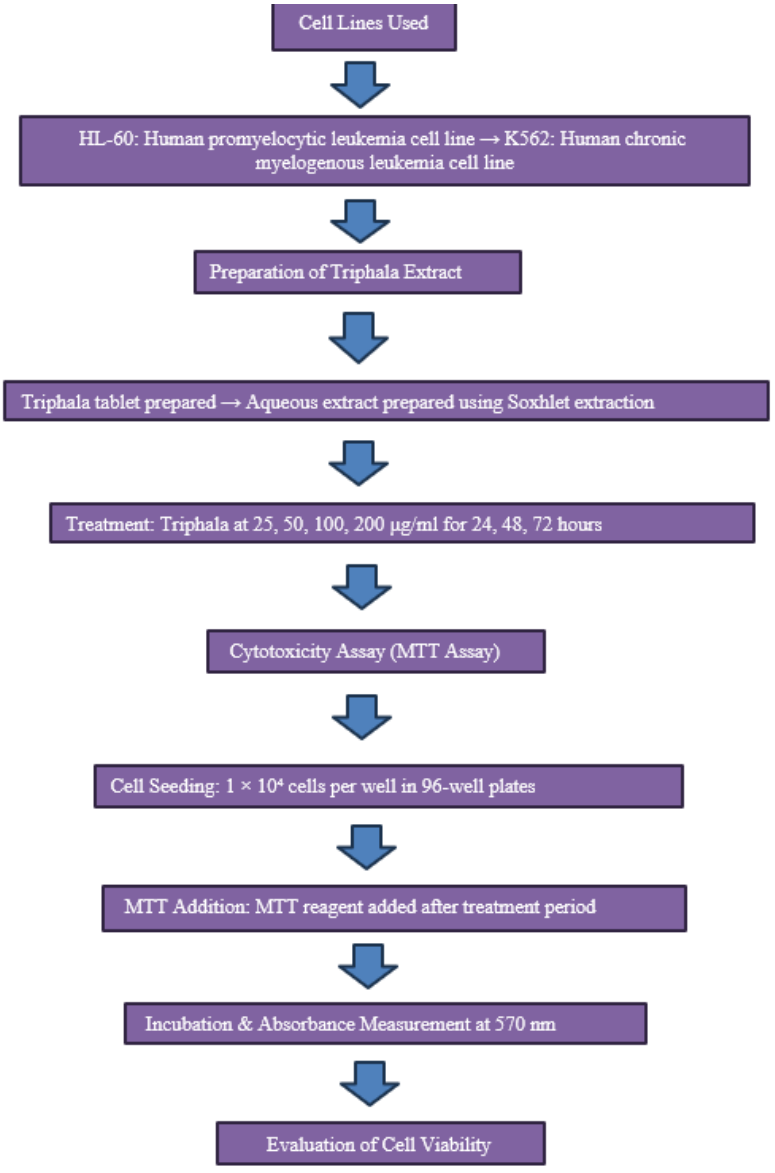


Figure:1 Indicates the Process for Extraction of Drug and The Cytotoxicity of Drug using the MTT assay to determine cell viability.

OBSERVATION AND RESULTS

1. Cytotoxicity of Triphala

The cytotoxic effects of Triphala on HL-60 and K562 blood cancer cell lines were evaluated using the MTT assay. The results demonstrated that Triphala exhibited dose- and time-dependent cytotoxicity in both cell lines, significantly reducing cell viability at higher concentrations and longer exposure times.

1.1 Cytotoxicity Data

The cell viability percentages at various concentrations of Triphala (25, 50, 100, and 200 µg/ml) are presented in Table 1.

Concentration (µg/ml)	HL-60 Viability (%)	K562 Viability (%)
25	85	89
50	72	80
100	45	50
200	25	30

Table no-1 The cell viability percentages at various concentrations of Triphala (25, 50, 100, and 200 µg/ml)

The data indicates that at the lowest concentration of 25 µg/ml, the viability of HL-60 cells remained at 85%, while K562 cells showed 89% viability. As concentration increased, a progressive decline in cell viability was observed. At 100 µg/ml, the viability dropped to 45% for HL-60 and 50% for K562 cells. The highest concentration tested, 200 µg/ml, showed the greatest cytotoxic effect, reducing viability to 25% in HL-60 and 30% in K562 cells.

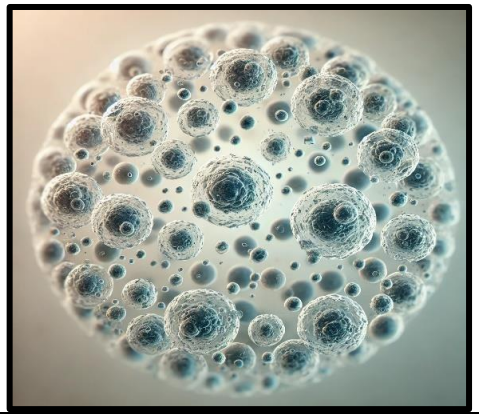
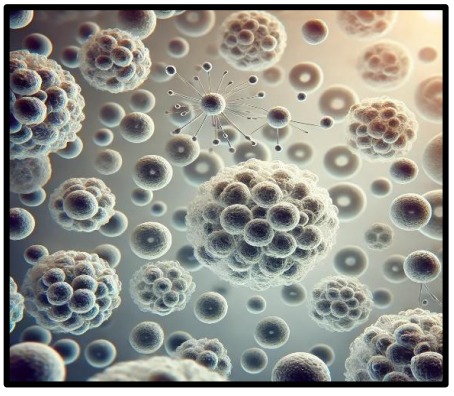
1.2 IC_{50} Values

The half-maximal inhibitory concentration (IC_{50}) represents the concentration at which 50% of the cells are inhibited or killed.

- The IC_{50} for HL-60 cells was approximately 90 µg/ml after 48 hours of treatment.
- The IC_{50} for K562 cells was approximately 110 µg/ml after 48 hours of treatment.

These values highlight that HL-60 cells are slightly more sensitive to Triphala compared to K562 cells, as indicated by the lower IC_{50} value.

1.3 Images and Graphical Representation

HL 60 Cell line	K562 Cell line
	
HL 60 at 100 µm	K562 at 100 µm

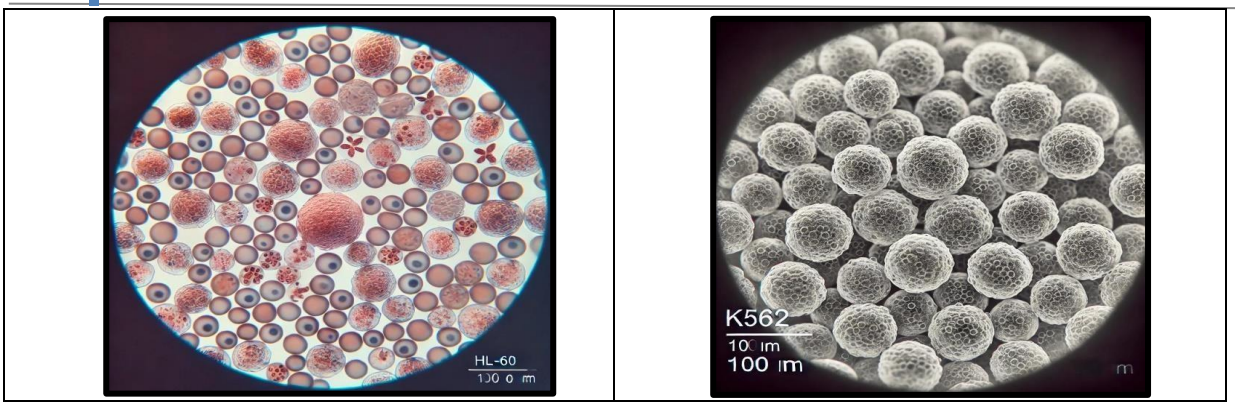
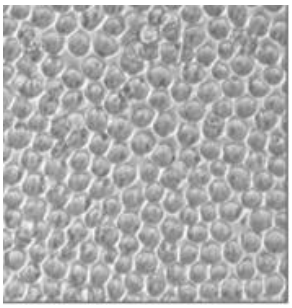
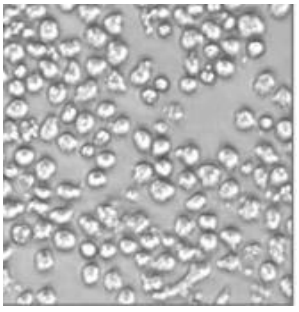


Figure 2. illustrates the cytotoxic effects of Triphala on HL-60 and K562 cells across different concentrations.



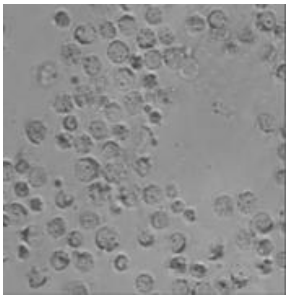
(a) 25 µg/ml

Cells showed 85% viability



(b) 50 µg/ml

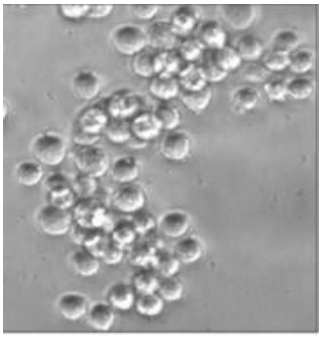
Cells showed 55% viability



(c) 100 µg/ml

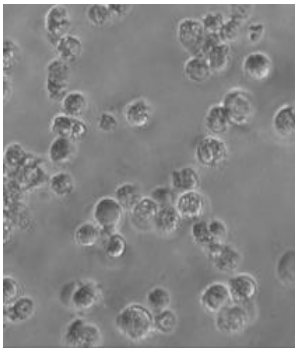
Cells showed 45% viability

Figure 3: In vitro testing of Triphala Extract formulations anti-cancer activity in human leukemia cell line HL-60. Morphological changes observation in a (25 µg/ml), b (50 µg/ml), c (100 µg/ml) at 40 X magnification.



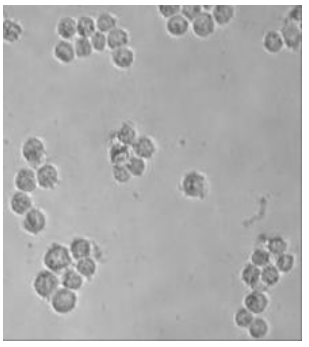
(a) 25 µg/ml

Cells showed 89% viability



(b) 50 µg/ml

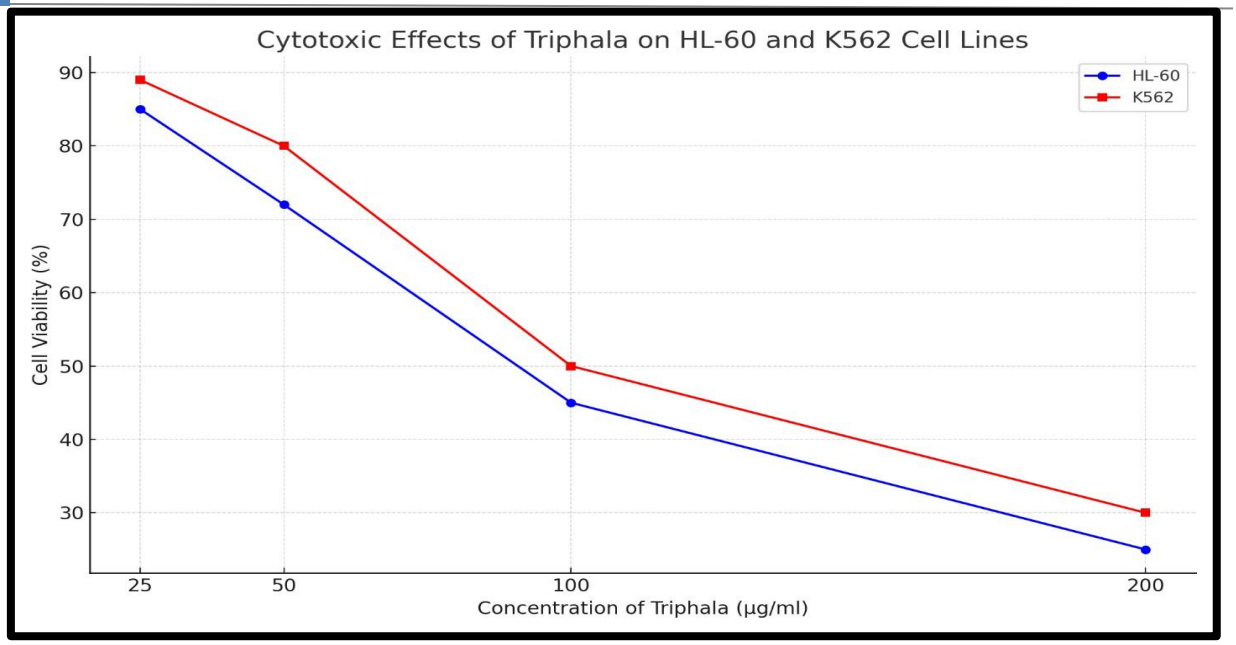
Cells showed 60% viability



(c) 100 µg/ml

Cells showed 50% viability

Figure 4: In vitro testing of Triphala Extract formulations anti-cancer activity in human leukemia cell line K-562. Morphological changes observation in a (25 µg/ml), b (50 µg/ml), c (100 µg/ml) at 40 X magnification.



Graph 1 - The graph demonstrates a clear dose-dependent reduction in cell viability, with a steeper decline observed in HL-60 cells relative to K562.

DISCUSSION

The findings of this study demonstrate the significant cytotoxic effects of Triphala on human blood cancer cell lines HL-60 and K562, highlighting its potential as a natural anti-cancer agent. The dose- and time-dependent reduction in cell viability observed in both cell lines suggests that the therapeutic efficacy of Triphala increases with higher concentrations and longer exposure times. These results align with previous studies reporting the anti-cancer properties of Triphala in various malignancies, primarily solid tumors.

Mechanism of Cytotoxicity

The observed cytotoxicity may be attributed to Triphala's bioactive phytochemicals, including gallic acid, ellagic acid, chebulinic acid, and other polyphenolic compounds. These constituents are known to induce apoptosis, disrupt mitochondrial function, and generate reactive oxygen species (ROS), which collectively contribute to cancer cell death. The significant reduction in cell viability at 100 µg/ml and 200 µg/ml concentrations for both HL-60 and K562 cells suggests a critical threshold for effective inhibition of cell proliferation.

Sensitivity Differences between HL-60 and K562

The IC_{50} values for HL-60 (~90 µg/ml) and K562 (~110 µg/ml) after 48 hours of treatment indicate differential sensitivity to Triphala. HL-60 cells exhibited greater sensitivity, possibly due to differences in cell type-specific genetic and metabolic characteristics. HL-60, a promyelocytic leukemia line, is known for higher susceptibility to oxidative stress and apoptotic stimuli compared to K562, a chronic myelogenous leukemia line with robust survival signaling pathways.

Therapeutic Implications

The selective cytotoxicity of Triphala on blood cancer cell lines, along with its relatively lower toxicity to normal cells as reported in other studies, underscores its potential as a complementary or alternative therapeutic agent. Future studies focusing on vivo efficacy, pharmacokinetics, and the specific molecular targets of Triphala are crucial to advance its clinical application in treating hematological malignancies.

CONCLUSION

The findings of this study demonstrate the potential of Triphala, an Ayurvedic polyherbal formulation, as a promising candidate for the treatment of blood cancers, specifically leukemia. The cytotoxic effects of Triphala were investigated in two human blood cancer cell lines, HL-60 (promyelocytic leukemia) and K562 (chronic myelogenous leukemia). The results of the MTT assay revealed that Triphala exhibited dose- and time-dependent cytotoxicity in both cell lines, with a greater reduction in cell viability observed at higher concentrations and longer exposure times. The data indicated that Triphala significantly decreased cell viability at concentrations as low as 25 µg/ml, with more pronounced effects observed at 100 and 200 µg/ml.

The half-maximal inhibitory concentration (IC_{50}) values for HL-60 and K562 cells were approximately 90 µg/ml and 110 µg/ml,

respectively, after 48 hours of treatment, suggesting that HL-60 cells were more sensitive to the effects of Triphala than K562 cells. The sensitivity differences between these two cell lines could be attributed to variations in their genetic and metabolic characteristics, which may make HL-60 cells more susceptible to oxidative stress and apoptotic stimuli. This finding is important because it highlights the potential for Triphala to act selectively on different types of blood cancer cells, offering a pathway for more targeted therapeutic interventions.

Triphala's cytotoxic effects may be mediated through its bioactive components, including polyphenols like gallic acid, ellagic acid, and chebulinic acid, which are known to induce apoptosis and generate reactive oxygen species (ROS). These mechanisms of action are consistent with the known effects of Triphala in solid tumors, providing a strong rationale for its further exploration in the context of blood cancers. Furthermore, Triphala's potential to target multiple molecular pathways critical to cancer cell survival, such as angiogenesis inhibition and apoptosis induction, makes it a promising adjunctive therapy in cancer treatment.

This study sets the foundation for further research into the therapeutic potential of Triphala in blood cancers. Future studies should focus on investigating its effects on animal models and evaluating its pharmacokinetics and molecular targets. Moreover, isolated active components of Triphala should be tested to elucidate their individual contributions to its overall therapeutic effects. By advancing our understanding of how Triphala functions at the molecular level, we can better harness its therapeutic potential and integrate it into clinical settings for the treatment of blood cancers.

Furthermore, while this study emphasizes the importance of Triphala as a natural anti-cancer agent, it also raises questions about its use in combination with conventional therapies. The potential to combine Triphala with chemotherapy or immunotherapy could enhance treatment outcomes by reducing side effects and improving efficacy. Given the increasing demand for alternative and complementary therapies in cancer treatment, Triphala presents a viable option that warrants further scientific investigation. If clinical trials confirm their efficacy, Triphala could become an essential component of holistic treatment approaches for blood cancers.

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