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Possible use of *Curcuma longa* extract as a post-chemotherapeutic supplement in acute myeloblastic leukaemia

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Abstract

Acute myeloid leukemia (AML) poses significant therapeutic challenges due to its intricate pathophysiology and the severe side effects associated with current treatments. Curcumin, a polyphenolic compound from *Curcuma longa*, is well known for its anti-inflammatory, antioxidant, and anticancer properties, yet its effects on hematological malignancies like leukemia remain insufficiently explored. This study investigates the impact of *Curcumin longa* extract on leukemia cells, focusing on cell viability, apoptosis, and morphological changes. Blood samples from AML patients were treated with various concentrations of the extract and incubated for different durations. The results demonstrated a dose- and time-dependent increase in leukemia cell destruction, with up to 88.89% cell death observed at higher extract concentrations and extended exposure times. Microscopic analysis revealed significant morphological changes, including nuclear condensation, cell membrane disruption, and other apoptosis-related features, indicating that curcumin induces apoptosis and inhibits leukemia cell proliferation. These findings suggest that curcumin possesses potent cytotoxic effects on leukemia cells and could serve as a valuable adjunct in leukemia therapy. Further research, including clinical trials, is necessary to evaluate the safety and efficacy of curcumin in leukemia patients and to explore potential synergistic effects with existing chemotherapy agents. This study delineates the potential of curcumin as a less toxic and more effective therapeutic option for leukemia.

Keywords: Acute myeloid leukemia, *Curcuma longa*, apoptosis

Introduction

Acute myeloid leukemia (AML) is marked by an elevated number of myeloid cells particularly blast cells in the bone marrow and a halt in their maturation. This often leads to hematopoietic insufficiency, manifesting as granulocytopenia, thrombocytopenia, or anemia, and may occur with or without leukocytosis [1]. Acute myeloid leukemia (AML) is the most prevalent type of acute leukemia in adults and is responsible for the highest number of leukemia-related deaths annually in the United States [2]. The typical treatment for AML encompasses induction chemotherapy, primarily comprising cytarabine combined with anthracycline. However, the survival duration for many patients undertaking conventional therapy is limited. Notably, the median survival for patients aged 65 years and older is only six months [3]. Despite notable advancements in leukemia treatment, conventional chemotherapeutic agents often demonstrate limited efficacy. The disease is associated with a poor prognosis, frequent relapses, and high mortality rates [4]. Curcumin (diferuloylmethane) is the natural yellow pigment found in turmeric, making up 3-4% of its composition [5]. It possesses a wide range of biological activities, including anti-inflammatory, antioxidant, anti-platelet, antiviral, anti-diabetic, and wound healing properties, along with its notable anti-carcinogenic effects [6, 7]. Phytochemicals which are the natural compounds derived from plants, are widely acknowledged as valuable sources for developing novel drugs. For example, curcumin, the primary polyphenol extracted from the rhizomes of *Curcuma longa*, has shown therapeutic benefits in various cancer types, including acute myeloid leukemia (AML) [8]. However, the underlying mechanisms of curcumin's action are complex and not fully understood, as it interacts with multiple targets and participates in various signaling pathways [9]. This study aims to investigate the cytotoxic effects of curcumin on AML cells, focusing on its ability to induce cell death and the resulting morphological changes.

Materials and Methods

Collection of Blood Samples from Patients with Acute Myeloblastic Leukemia

Blood samples from patients diagnosed with acute myeloblastic leukemia (AML) were collected by qualified laboratory professionals. This study was conducted following stringent ethical guidelines to ensure the integrity and confidentiality of patient information. These samples were treated with EDTA to prevent coagulation and were subsequently processed within a controlled laboratory environment. To maintain confidentiality and protect patient privacy, no personal identifiers were recorded or mentioned in this manuscript.

Preparation of Fresh Curcumin Extract from *Curcuma longa* Rhizomes

Fresh *Curcuma longa* rhizomes were harvested and thoroughly washed to remove any soil or debris. Under sterile conditions, a scalpel cut the rhizomes into small pieces, ensuring precision and consistency. These pieces were then transferred into a Falcon tube. Subsequently, 10 mL of sterile saline solution was added to the tube to facilitate the extraction process. The tube containing the *Curcuma longa* pieces and saline solution were vigorously vortexed to ensure thorough mixing and to facilitate the release of bioactive compounds into the saline. Following vortexing, the mixture was subjected to centrifugation at 12,000 rpm for 5 minutes. This high-speed centrifugation step was crucial to separate the solid debris from the liquid extract efficiently. After centrifugation, the supernatant, which contains the soluble compounds of interest, was carefully collected using a micropipette and transferred into sterile Eppendorf tubes. These aliquots of the supernatant were then stored under appropriate conditions until they were required for subsequent experimental analyses. The collected supernatant was then used in various biochemical and cellular assays to investigate the potential anticancer properties of *Curcuma longa*, with a particular focus on its effects on acute myeloid leukemia (AML) cells.

Preparation of Control Samples for Comparative Analysis in Acute Myeloid Leukemia Studies

To establish a baseline for comparative analysis and ensure the validity of our experimental results, a control sample was meticulously prepared. This control was designed to match the experimental conditions as closely as possible, except for the absence of curcumin extract, thus specifying the effects of curcumin on acute myeloid leukemia (AML) cells.

Initially, 100 microliters of EDTA-treated blood were aliquoted into a sterile Eppendorf tube. This volume was consistent with the experimental setups to maintain uniformity across all samples. To this aliquot, 50 microliters of sterile saline solution were added. The control sample was then gently mixed to ensure thorough homogenization of the blood and saline. This mixing step was critical to mimic the treatment process applied to the curcumin samples and ensure that any observed effects in the experimental groups could be attributed specifically to the curcumin extract. Subsequently, the control sample was subjected to the same incubation periods as the experimental samples, which were 10 minutes, 30 minutes, and 60 minutes. This parallel incubation allowed for direct comparison between the control and curcumin-treated samples at each time point. This control setup was pivotal in

distinguishing the specific impact of curcumin from other potential variables, thereby strengthening the validity of our experimental findings and contributing to a more comprehensive understanding of curcumin's therapeutic potential in the treatment of acute myeloid leukemia.

Treatment of Patient-Derived Acute Leukemia Blood Samples with Curcumin Extract in Differing Test Conditions

In this study, we utilized EDTA-treated blood samples obtained from patients diagnosed with acute myeloid leukemia (AML) to investigate the effects of curcumin extract on leukemic myeloblasts (Fig.1). Two different concentrations of curcumin extract were prepared and tested across multiple time intervals to evaluate their impact on the leukemic cells. Initially, 100 microliters of EDTA-treated blood were aliquoted into separate Eppendorf tubes. In the first set of experiments, 10 microliters of curcumin extract were added to one of these Eppendorf tubes, resulting in a final volume of 110 microliters. In a parallel setup, another 100 microliters of EDTA-treated blood were combined with 50 microliters of curcumin extract, resulting in a total volume of 150 microliters in the second Eppendorf tube. This process was meticulously repeated to ensure precision and consistency. To assess the temporal effects of curcumin on AML cells, each experimental setup was incubated for three different periods: 10 minutes, 30 minutes, and 60 minutes.

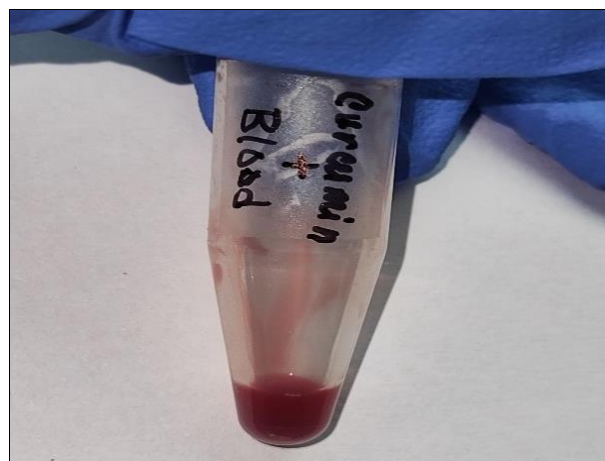


Fig 1: Blood sample 100 microlitre is mixed with 10 microlitres of curcumin extract

Preparation of Blood Smears for Microscopic Analysis

To facilitate detailed microscopic examination of the treated and control blood samples, we prepared blood smears following a meticulous protocol to ensure consistency and accuracy across all samples. Initially, clean glass slides were selected, and each slide was thoroughly cleaned to remove any potential contaminants that could interfere with the analysis. This cleaning step was crucial to ensure that the slides were free from any residues that might affect the staining process or microscopic observation. After the incubation periods (10, 30, and 60 minutes) for both curcumin-treated and control samples, 10 microliters of blood from the respective Eppendorf tubes were carefully pipetted onto the cleaned glass slides. Using a spreader slide, the blood was gently and evenly spread across the surface of the glass slide. This technique was executed with precision to create a uniform smear, essential for subsequent

staining and microscopic analysis. Once the blood had been spread, the slides were left to air dry completely. The drying process was monitored to ensure that no moisture remained, as this could impact the quality of the staining. Upon drying, the slides were stained using Leishman stain, a commonly used Romanowsky stain for differential staining of blood smears. The staining process was conducted as per the required protocol. After the staining process was completed and the slides had dried, they were examined under a light microscope. The microscope was calibrated to the appropriate magnification to observe the morphological characteristics of the blood cells. This examination included assessing cell morphology, identifying apoptotic cells, and evaluating any other cellular changes induced by curcumin treatment.

Results

Our study revealed a significant impact of curcumin on myeloblastic cells derived from patients with acute myeloid leukemia (AML). The curcumin treatment induced notable cell cycle arrest and apoptosis in these myeloblastic cells, with the extent of cellular destruction increasing proportionally with both the concentration of curcumin and the duration of exposure. Initial observations indicated that the treatment with curcumin led to a discernible reduction in the number of viable myeloblastic cells. Specifically, when 10 microliters of curcumin extract were added to 100 microliters of EDTA-treated blood and incubated for 10 minutes, the percentage of destroyed myeloblastic cells was observed to be 23.52%. This indicated a substantial early effect of curcumin, even at lower concentrations and shorter exposure times.

Further analysis demonstrated that extending the duration of exposure and increasing the concentration of curcumin significantly amplified its cytotoxic effects. When the same volume of blood (100 microliters) was treated with a higher concentration of curcumin extract (50 microliters) and incubated for 60 minutes, the percentage of destroyed

myeloblastic cells escalated dramatically to 88.89% (Table 1, Fig. 2). This pronounced increase underscores the dose- and time-dependent nature of curcumin's cytotoxic effects on AML cells.

Morphological Changes Observed in Myeloblastic Cells Treated with Curcumin Extract

Upon microscopic examination, a range of morphological changes were observed (Fig.3) in the myeloblastic cells treated with curcumin extract, indicating significant cellular responses to the treatment. These changes provide insights into the mechanisms of curcumin-induced cytotoxicity and its potential as a therapeutic agent for acute myeloid leukemia (AML).

One prominent change was the condensation of the nucleus in many cells. This nuclear condensation is indicative of the early stages of apoptosis, a programmed cell death pathway. The presence of such morphological features suggests that curcumin induces apoptosis in myeloblastic cells, thereby inhibiting their proliferation and survival. Additionally, a number of cells exhibited disrupted cell membranes, leading to the leakage of intracellular contents. This membrane disruption signifies a loss of cell integrity and subsequent cell death, a process often associated with necrosis. The release of cellular contents into the extracellular space is a hallmark of necrotic cell death. Interestingly, some cells displayed intact cell membranes but lacked a visible nucleus. The absence of a nucleus in these cells may indicate karyolysis, where the nuclear material is dissolved, or an advanced apoptotic stage where the nucleus has been degraded. In contrast, other cells showed intact nuclei with disrupted cell membranes. This phenomenon suggests that while the nuclear material remains preserved, the structural integrity of the cell membrane is compromised. Such cells are likely in a transitional phase between apoptosis and necrosis, reflecting the complexity of curcumin's action on cellular structures.

Table 1: Table showing the percentage of leukemia cells being destroyed when treated with curcumin extract

Time of leukemia cells + curcumin extract exposure(mins)	Quantity of curcumin extract added	Percentage of Myeloblastic cells destroyed
10 mins	10 microlitre	23.52%
10 mins	50 microlitre	35.71%
30 mins	10 microlitre	50%
30 mins	50 microlitre	66.67%
60 mins	10 microlitre	71.42%
60 mins	50 microlitre	88.89%

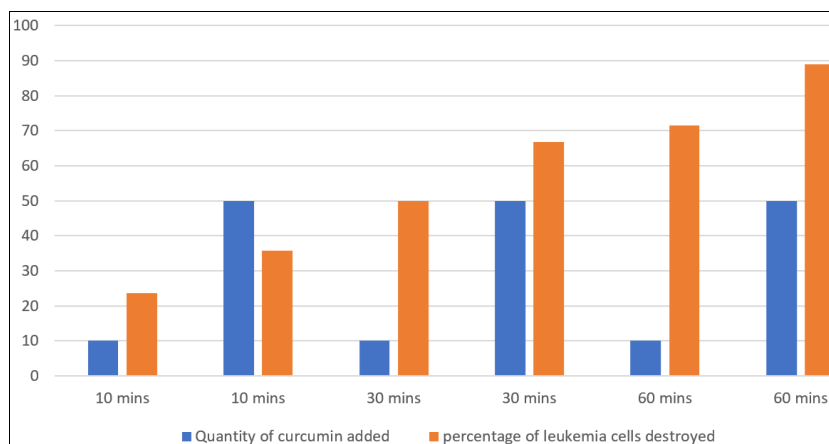

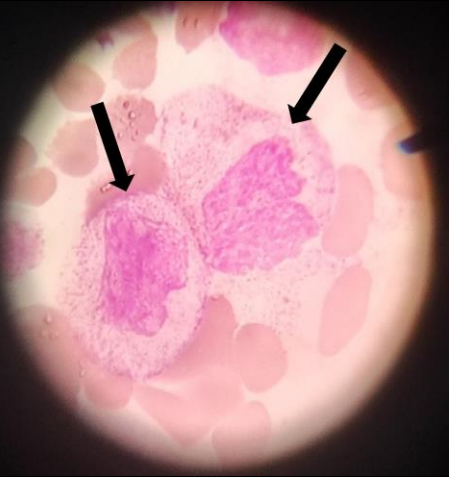
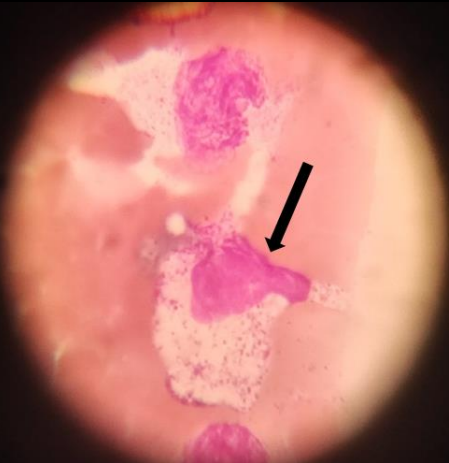
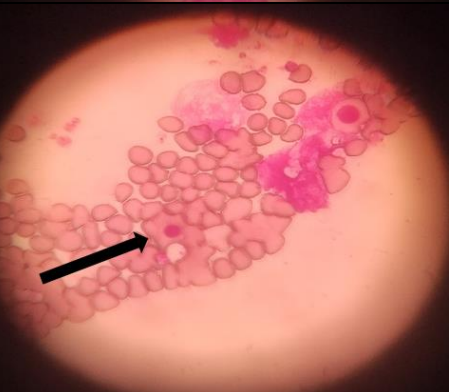


Fig 2: Effect of Curcumin extract on Leukemia Cell Destruction

Morphological changes observed	Image under microscope
<p>Large dark pink coloured cell is the myeloblastic cell in the control setup</p>	
<p>Myeloblastic cell found in the control setup</p>	
<p>The cell membrane of the myeloblasts is destroyed and cell contents moved out</p>	
<p>The condensation of the nucleus, indicative of pre apoptotic stage</p>	

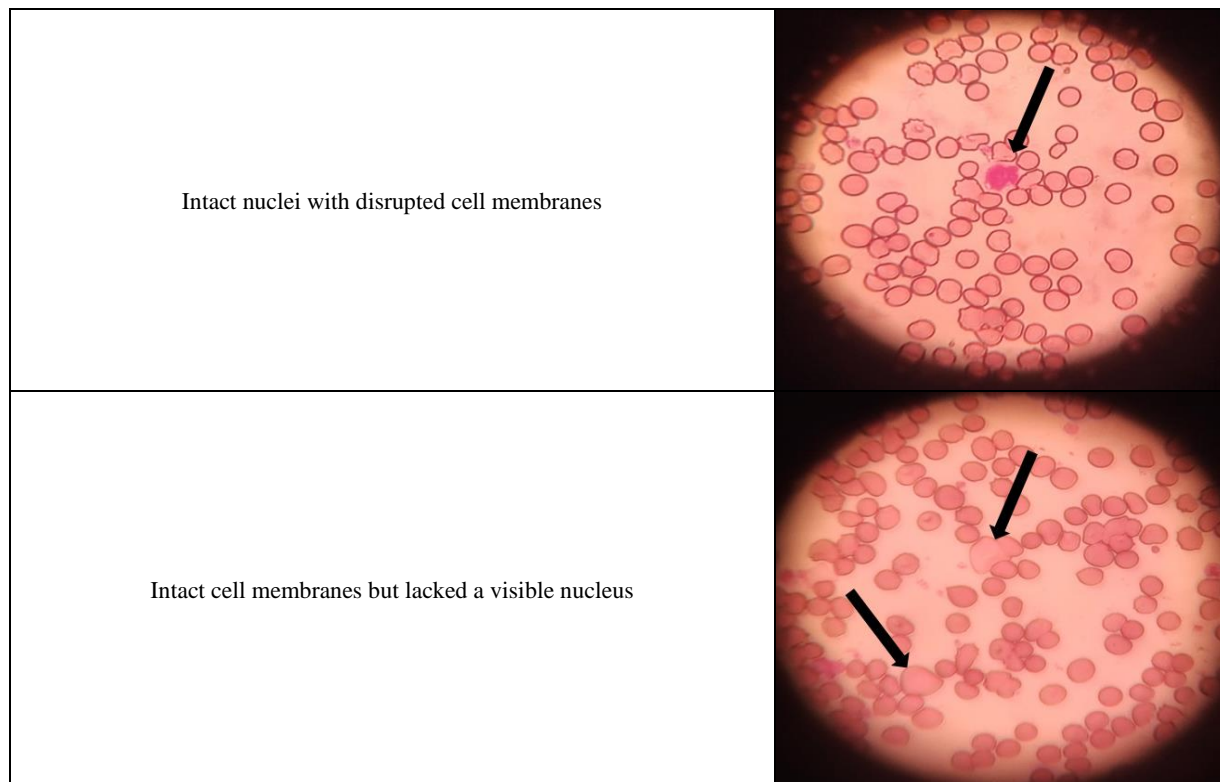


Fig 3: Microscopic observation of the Morphological Changes in Leukemia Cells Treated with Curcumin Extract

Discussion

Curcumin, a bioactive compound extracted from the rhizomes of *Curcuma longa*, has garnered significant attention for its potential anticancer properties across various malignancies, including acute myeloid leukemia (AML) [10].

In our study, we investigated the cytotoxic effects of curcumin on myeloblastic cells derived from AML patients. Our experimental design involved exposing these cells to varying concentrations of curcumin extract over different time intervals to assess the dose-response relationship and temporal dynamics of cell death. Our findings revealed a marked increase in the cytotoxic efficacy of curcumin with both higher concentrations and longer exposure times. Specifically, the cell viability assays indicated that curcumin exposure led to a significant reduction in the survival of myeloblastic cells, achieving an impressive 88.89% cell death at optimal conditions. Also, we have observed several morphological changes in the myeloblastic cells which are indicative of their destruction due to the effect of curcumin.

Previous research has demonstrated that curcumin significantly decreases the survival and proliferation of AML cells *in vitro* [11]. Moreover, curcumin has shown efficacy in reducing AML cell proliferation within hematopoietic tissues and limiting their dissemination into non-hematopoietic tissues. In a comparative study evaluating the cytotoxic effects of four phytochemicals—curcumin, epigallocatechin gallate (EGCG), genistein, and resveratrol—curcumin emerged as the most potent agent against AML [12]. This comparative analysis revealed that curcumin exhibited the strongest anti-AML efficacy, surpassing the cytotoxic effects of the other phytochemicals tested.

Conclusion

In conclusion, our research aligns with previous findings and offers a detailed analysis of curcumin's significant anti-

AML properties. Curcumin's capacity to trigger apoptosis, suppress cell proliferation, and hinder the spread of AML cells underscores its potential as a therapeutic agent. Future studies should prioritize clinical trials to evaluate the safety and effectiveness of curcumin in treating AML patients. This study highlights the promise of curcumin as a potent weapon in the battle against acute myeloid leukemia,

Conflict of Interest: The authors declare no conflict of interest.

Author's Contribution: Dr. Satadal Das designed the study procedure. Mr. Arijit Halder and Ms. Nikita Parui carried out the experiment, analyzed the data, and wrote the manuscript. Dr. Sugat Sanyal and Dr. Satadal Das reviewed and edited the manuscript.

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