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Original research article

CYTOTOXICITY OF ALOE VERA EXTRACT ON CHRONIC MYELOID LEUKEMIA CELLS; IN VITRO STUDY ON CYTOTOXICITY ON HL-60 CELLS

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Abstract: *Aloe vera has been traditionally used for its various therapeutic properties, but its potential anticancer effects have not been thoroughly explored. Understanding its efficacy against leukemia could significantly impact care recommendations for leukemia patients.* ***Objective:*** *This in-vitro study aims to establish the anti-cancerous effects of Aloe vera on the Human Leukemia HL-60 cell line.* ***Methods:*** *Following approval from the Ethical Review Committee, this study involved culturing the HL-60 cell line and treating it with increasing concentrations of Aloe vera extract to determine the half-maximal inhibitory concentration (IC50). The MTT assay was employed to assess cell viability, and the absorbance was measured using an ELISA reader. Gene expression levels of pro-apoptotic biomarkers, Caspase-3 and Caspase-9, were also quantified to gauge apoptosis induction.* ***Results:*** *Aloe vera demonstrated a relatively low IC50 value of 13.1 µM in the HL-60 cell line, indicating potent cytotoxicity. Notably, pro-apoptotic biomarkers were highly expressed, with Caspase-3 and Caspase-9 recording gene expression levels of 11.1 and 13.7, respectively.* ***Conclusion:*** *Aloe vera exhibited significant pro-apoptotic effects against the leukemia HL-60 cell line, suggesting its potential as an anti-cancer agent. Further in-vivo and clinical studies are warranted to elucidate the specific pathways through which Aloe vera exerts these effects and to validate its use in clinical settings.*

Keywords: Aloe Vera; Anti-Cancer Agents; Apoptosis; Caspase-3; Caspase-9; HL-60 Cells; Leukemia; MTT Assay.

Introduction

Chronic Myeloid Leukemia (CML) is a neoplastic disease of the hematopoietic system in which there is a clonal expansion of the myeloid cells in the bone marrow and peripheral blood and most commonly results from a BCR-ABL1 translocation (1). Several challenges remain unaddressed even after the development of targeted therapies including the tyrosine kinase inhibitors (TKIs); these barriers include TKI resistance and side effects this calls for the discovery of other treatment modalities (2). Traditionally used natural products have recently attracted much attention due to their traditional medicinal properties including inhibitory activity against cancer cells. Aloe vera is a commonly used plant and has become the subject of research interest because of its medicinal activities that include; anti-inflammatory, antioxidant and anti-cancer activities (3).

Aloe vera has several biological active ingredients, such as polysaccharides, glycoproteins, and phenolic compounds, all of which have evident anti-cancer effects (4). It has been reported that Aloe vera has a pro-apoptotic effect in several cancer cell lines and works to inhibit carcinogenic activity (5). Another necessary process associated with cancer therapy, apoptosis, is the type of cell death modeled according to preprogrammed genetic instructions; it is appropriate for cancer cells because they do not affect normal cells (6). The study of the efficiency of Aloe-vera in eliciting apoptosis in leukemia cells such as HL-60, which is a model system employed in the research of APL, acute promyelocytic leukemia, has been the focus of the scientific community (7).

HL-60 cells were derived from the bone marrow cells of a patient with acute promyelocytic leukemia; this cell line has higher proliferative activity and is highly resistant to apoptosis, hence its use in assessing the anti-cancer activity of some agents (8). Aloe vera used in this regard is intended to determine the ability of the plant to cause apoptosis and possibly overcome some resistance mechanisms present in leukemia cells (9). Earlier experiments have shown that Aloe vera extracts can affect cell survival and apoptosis signaling pathways, such as the activation of caspases, which are known to control the apoptosis signals (10). Thus, the present work aims to ascertain the cytotoxic action of Aloe vera extracts on the HL-60 cell line with a special reference to the pro-apoptotic effect. The findings could help design new treatment approaches, including managing leukemia that has not responded well to standard therapies.

Methodology

This four-month (November 2022- March 2023) inter-collaborative work was carried out in cell culture Laboratories in affiliated institutes after approval by the concerned department. Aloe was extracted from the plant directly without purifying it first. In detail, crude extract

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from the collected plant parts was frozen and then ground into fine powder under aseptic conditions and subjected to freeze drying process under -55℃ using a freeze dryer. The concentrations for the treatment of cells were 10, 20, 40, and 60 (µg/ml), and these were prepared from fresh Gibco medium (Dulbecco's Modified Eagle Medium, DMEM #11977055) with the recommended supplements for the treatment of cells. HL-60 cells were preserved in specific Cryo vial preparations maintained in Liquid Nitrogen at -180°C. Following thawing and adherence to standard subculturing techniques, the cells were cultured in a supplemented medium containing 10% FBS. The phytochemical Aloe V. was administered in pre-calculated concentration ratios of 1.5, 2, 2.5, and 3 µM concentrations, and the MTT assays were taken at 24, 48, and 72hrs respectively. Only 24 hours of MTT were used to determine IC50 during the experiments because of the higher experimental reproducibility. Total RNA was extracted from treated cells, and a quality check (ng/µl) of RNA was done per the kit protocol. The extracted RNA was then used to synthesize cDNA based on the protocol of the cDNA synthesis kit (Catalog #AM7811, Thermofisher Scientific, USA)—primer sets of Caspase-3 and Caspase-9 genes. Primers were synthesized with the help of a serial cloner by using the consensus CDS sequence of required NCBI and In-Silico PCR genes at the UCSC Genome Browser.

Results

The HL-60 cells were exposed to decreasing dilutions of Aloe Vera extract, and the toxicity rate was measured using an MTT assay to determine the HL-60 IC50 values. Out of a total of 24, 48, and 72 hours assays, because of a higher degree of reproducibility of MTT assay values, only the 24-hour MTT assay was selected for proceeding with the best IC50 of Aloe V. (IC50 of 13. 7uM) treatment concerning Cisplatin (Control). The quantitative gene expression of Caspase 3 and 8 was done to access pro-apoptotic. The high correlation is signified by the higher relative gene fold, demonstrating the effectiveness of compounds on HL-60 cells, as indicated in Figure 1.

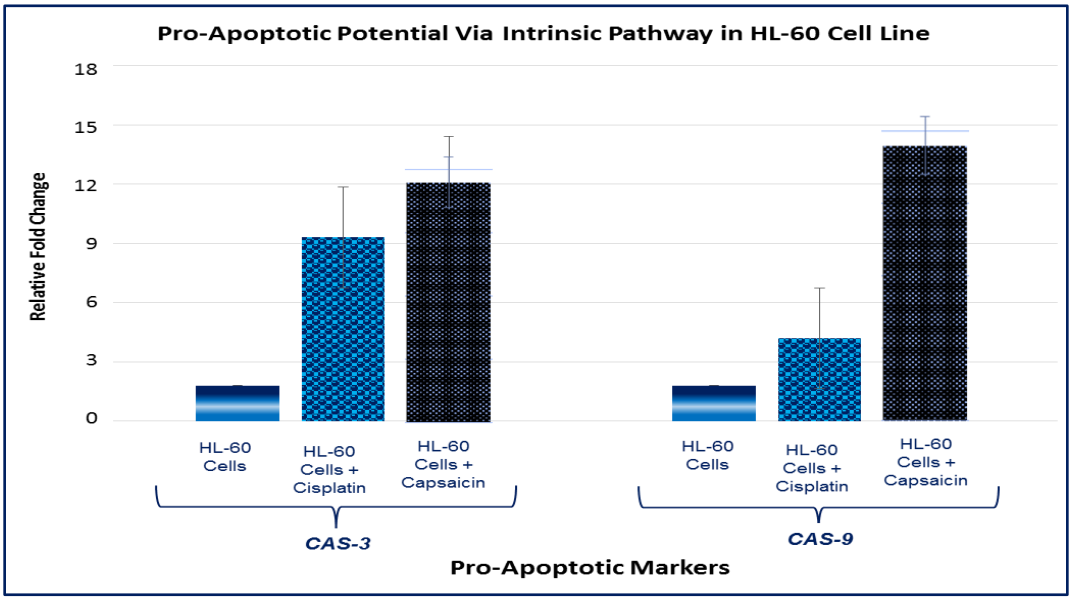

Figure 1: Expression Profiling of Caspase-3 and Caspase-9

Both the apoptotic markers (Caspase-3 and Caspase-9) are augmented in the treated cells as compared with control cells, and cells treated with Aloe V. Caspase-9 gene expression was observed as 14. One overall relative gene fold, which was significantly higher than Caspase-3 11. 7.

Discussion

Aloe vera, as a medicinal plant, has been known for many years to inhibit the initiation and promotion of cancer. Such properties as anti-oxidative, anti-inflammatory, pro-apoptotic, anti-proliferative, and anti-tumorigenic effects have been proved in multiple cancer cell lines (11). The present work shows that Aloe vera extract has a cytotoxic effect on HL-60 cells, equivalent to CML. The treatment also enhanced the levels of Caspase-3 and Caspase-9 genes, proving the contention that Aloe vera compounds induced intrinsic apoptosis that may be the reason behind the deceleration of proliferation in cancerous cells (12). These results correlate with earlier studies, for example, where piperine was reported to have a genotoxic effect in MCF-7 cell lines and caused apoptosis through activation of Caspase-3 and Caspase-8 (13).

The present investigation also indicates the effectiveness of Aloe vera at comparatively lower concentrations in inhibiting cancerous cell formation. Another study pointed out that a concentration of 8 µM application of Aloe vera affects breast cancer cells' proliferation process and decreases their viability and interactive impact upon the MMPs, which play a crucial role in the metastatic process (14, 15). In agreement with this observation, we have shown here that Aloe vera hits apoptosis and cuts the cell progress of HL-60, and this impact could be generalized to different sorts of cancers. However, in comparing the present study's findings with those of other related studies, some other natural compounds were revealed, such as cinnamaldehyde. In another study, employing flow cytometric examination of leukemia cells treated with the IC50 concentration of

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cinnamaldehyde and Aloe vera, changes in size as well as in the granularity of the cells were observed, explaining altered cellular structure and cellular integrity (16). This disruption of cellular metabolic machinery favors the idea that Aloe vera enhances membrane permeability to facilitate cell death by rupturing cell membranes and defragmentation of interior organelles. Similar findings were concluded in experiments on HepG2 liver cancer cell lines to support Aloe vera's anti-cancer characteristics of a broad category (17).

Thus, the peculiarity of the Aloe vera action is linked with the destruction of cancer cell proliferation and stimulation of apoptosis. For instance, a fluorescence microscopy study with Hoechst 33258 dye revealed that Aloe vera application leads to apoptotic body formation and a conspicuous degree of nuclear fragmentation, suggesting that Aloe vera induces apoptotic cell death (18). In the extension to this research and to enrich the integrity of experiments, the technique of High-Performance Liquid Chromatography HPLC is recommended to extract the targeted phytochemical from the clove oil as a complete extract in the form of oil that can contain several anti-cancer components. Furthermore, the molecular mechanism of action of Aloe V. with other anti-metastatic and anti-proliferative biomarkers must be studied.

Conclusion

Our studies supported the reports regarding the pro-apoptotic effects of Aloe V. on the HL-60 cells. They also pointed out that Aloe V. was beneficial in offering good chemoprotection. The effects observed among cancer cell howling include disruption of the cellular metabolic machinery and induction of apoptosis, hence justifying the need for Aloe V. to be used as an adjuvant therapy. The results of these studies suggest a need for further clinical trials and investigations of Aloe V. to be used in cancer patients.

Declarations

Data Availability statement
All data generated or analyzed during the study are included in the manuscript.
Ethics approval and consent to participate.
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Conflict of interest

The authors declared an absence of conflict of interest.

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