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ORIGINAL RESEARCH **PERIOPERATIVE MEDICINE**

Establishing capsaicin's anti-cancer intricacies in chronic myelogenous leukemia care: insights from human K562 cells

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ABSTRACT

Objective: We aimed to establish capsaicin as a potent natural chemotherapeutic agent in chronic myeloid leukemia by unveiling anti-cancer mechanisms, concentrating at pro-apoptotic and anti-proliferative effects on K562 cells.

Methodology: After REC approval, this research proceeded with culturing K562 cells and treatment with serial concentrations of capsaicin for calculation of subsequent 50% Inhibitory Concentration (IC50) values via MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay, expression analysis of gene, comparative analysis of relative gene fold (intrinsic biomarkers caspase-3, caspase-9) as apoptosis mediator biomarkers followed by Quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR). Anti-proliferative activity was confirmed via Nitric Oxide (NO) releasing Assay.

Results: For K562 cells, the IC50 of capsaicin (12.1 µM) showed high gene expression of intrinsic pro-apoptotic biomarkers caspase-3 and caspase-9 with the relative gene fold of 12.1 and 13.9 respectively. Persistent peaks of NO levels confirmed the anti-proliferative potential of the compounds. Strongest growth inhibitory activity was seen at the peak time of 100-120 min.

Conclusion: Capsaicin proved to be a strong pro-apoptotic and anti-proliferative phytochemical agent leading to therapeutic effects against K562 cells. Further studies would help in identifying key molecular intricacies by which capsaicin exhibits diversified anti-cancer potential.

Abbreviations: CML - chronic myelogenous leukemia; NO - Nitric oxide; ROS - Reactive Oxygen Species; ROS - Reactive Oxygen Species

Keywords: Capsaicin, Pro-apoptotic Effects, Anti-Cancer Mechanisms, K562 cells, Natural Chemotherapeutics

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1. INTRODUCTION

Leukemia is a blood malignancy that presents some big challenges, since new chemotherapeutic agents are aggressive and can result in poor patient outcomes and long-lasting adverse effects.[1] An increasing interest exists in utilizing natural phytochemicals to lessen the drawbacks of traditional cancer treatments.[2] Exploring the anticancer activities of dietetic phytochemicals has been the subject of growing interest in the recent decades.[3] These organic substances may help to supplement or improve the effectiveness of current chemotherapy treatments. This strategy is especially important for those with chronic myelogenous leukemia (CML), because controlling the illness and reducing toxicity from therapy are the top priorities.[4]

Among these natural substances, phytochemicals derived from plants and phenylpropanoids found in essential oils have demonstrated potential in halting the formation of tumors and triggering apoptosis in leukemia cell lines like K562.[5] For example, capsaicin has been shown to induce apoptosis in K562 cells via processes involving Reactive Oxygen Species (ROS) and regulation of signaling pathways essential for the survival of cancer cells. [6] Concentrated in the seeds and white pith of chili peppers (Capsicum annum), capsaicin is a powerful apoptosis inducer with a long history of medical use, including the treatment of cancer, diabetes, asthma, arthritis, and gastrointestinal issues. [7] Capsaicin treatment damages DNA, affects DNA repair systems and disrupts the metabolism of cancer cells. Both substances have been employed in pharmacology and medicine for a long time because of their multidirectional potential. Numerous tumor cell lines have demonstrated anticancer action, and capsaicin has a strong apoptotic potential. [8]

Nitric oxide (NO) plays a dual role in pathophysiology, acting as both a signaling molecule and a mediator of cellular damage. In normal physiology, NO is crucial for vasodilation, neurotransmission, and immune response. However, in pathological conditions, excessive NO production can lead to oxidative stress, DNA damage, and apoptosis, contributing to inflammatory diseases, neurodegeneration, and cancer. [9] According to research, capsaicin causes K562 cells to undergo apoptosis by causing the generation of reactive oxygen species (ROS) and altering vital signaling pathways that are necessary for the survival and growth of cancer cells. The promise of capsaicin as a targeted treatment against leukemia and maybe other malignancies is highlighted by its dual action. [10]

Furthermore, plant-based phytochemicals were proven to be very bioactive against cancer cell lines like HeLA cells. [11] It has been demonstrated that these substances can stop the cell cycle and reduce angiogenesis; this is especially true when combined with more established chemotherapy drugs like cisplatin. Cisplatin is a benchmark used in MTT assays to assess the anticancer and antiproliferative qualities of natural phytochemicals. Cisplatin is well-known for its efficacy in treating a variety of malignancies. [12] .This strategy highlights the significance of looking into natural sources for cutting-edge therapeutic molecules in cancer while also extending the range of available therapy alternatives. [13]

We aimed to establish capsaicin as a potent natural chemotherapeutic agent in CML by unveiling anti-cancer mechanisms concentrating at pro-apoptotic and anti-proliferative effects on K562 cells.

2. METHODOLOGY

After REC approval (143-2024), this study involved Authors' affiliated institutions based ICMJE contribution and research laboratories at; Liaquat University of Medical & Health Sciences (LUMHS), Jamshoro, Bilawal Medical College Liaquat University of Medical and Health Sciences, Jamshoro, Mirpur University of Science and Technology (MUST), Mirpur (AJK), Bhitai Medical & Dental College, Mirpurkhas, and Forman Christian College, Lahore. Capsaicin was extracted from the powdered seeds (freeze dried) under hygienic conditions and white pith of chili peppers (Capsicum annum). Dulbecco's Modified Eagle Medium was used for the treatment of cells, customized concentrations of 10, 20, 40 and 60 µg/ml, were freshly prepared Gibco (Dulbecco's Modified Eagle Medium) DMEM (catalog#11966011 USA) medium supplemented (or enriched) with L-glutamine (2 mM), 10% fetal bovine serum (FBS), sodium pyruvate (1%), penicillin and streptomycin (100 IU/mL) and vortex for 10-12 min.

K562 cells were initially obtained from commercially available sources (Thermosfisher certified) and controls were also extracted from leukemic blood samples and stored in pre-defined Cryo vials preparations in liquid nitrogen at −180ºC. After thawing and following routine sub culturing protocols, cells were cultured in supplemented Medium (10% Fetal Bovine Serum) and treated with pre-formulated concentrations of capsaicin (05, 10, 15, 20, 25, and 30 µM) for MTT assays of 24, 48, and 72 hours, respectively. Cells (10000 cells/well) were seeded in 96-well plates (100 µL media/well) and treated with serial dilutions of Capsaicin (10, 20, 40 and 60 µg/ml).

After reproducing the experiments, as per greater reproducibility in standard practice with all SOPs, only 24hours MTT was selected for IC50 calculations. IC50

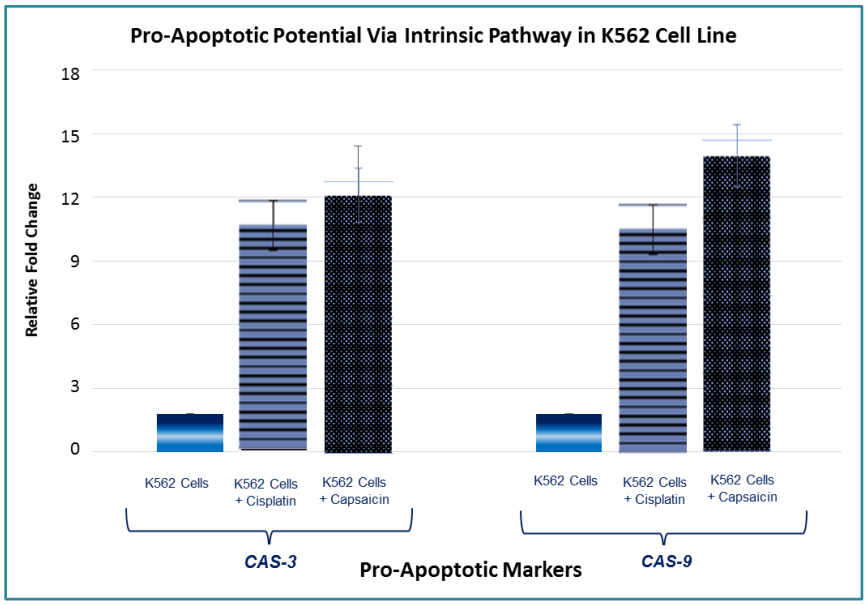

Figure 1: MTT assay (expression profiling of selected biomarkers)

is the concentration at which approximately 50% cells were found dead). From treated cells, total RNA extraction was performed followed by Quality check (ng/µl) and cDNA synthesis as per Kit Protocols (Reagent Catalog# AM7832, Thermofisher Scientific, USA). Primers were designed on serial cloner by taking the consensus CDS sequence of required genes from NCBI and confirmed by Doing In-Silico PCR on UCSC Genome Browser and external control was applied. Gradient PCR reactions were done multiple times to achieve optimization within Tm of 61.4-62.1°C.

To perform RT-qPCR for the expressional analysis of the Caspase-3 and Caspase-9, CFX 96 qPCR system by Bio-Rad was used and Manufacturer's protocol was followed (Catalogue # M0241, Thermo Fisher Scientific, USA). The normalizing IC (Internal Control) was a housekeeping gene ACTB (β-Actin). To calculate the difference in expression of the biomarkers with reference to the control, the following formula was applied,

$$\mathbf{\Delta}C_T = C_T \text{ (Test)} / C_T \text{ (Calibrator)}$$

Where, $\mathbf{\Delta}C_T$ represents the differential expression of the biomarker in the C_T value of the biomarker in control (Test) and the C_T value of the biomarker in the sample. GraphPad Prism Software, Version 9, and Microsoft Excel were utilized to carry out statistical analysis based on relative gene fold and other calculations (considering a p-value of ≤ 0.05 as statistically significant). For Colorimetric assay a metabolic kit (E-labscience Lot#48T/94T, USA) was used as per manufacturer's instructions. After treatment of K562 cells with IC50 of Capsaicin, the NO levels were determined. Along with 1M sodium nitrite solution and concentrations (uM) of Control (Cisplatin), nitrite absorbance (520nm) versus concentration curve was prepared. All calculations and handling of Experiments were reproduced twice to ensure credibility of work along with Quality Controls (Blank, negative and positive control).

Statistical Analysis: Data were analyzed using SPSS version 26.

3. RESULTS

3.1. Chemo-Preventive Potential (CPP) Testing: In Vitro MTT Assay of Capsaicin on Human K562 Cell line:

K562 cells were treated with serial concentrations of capsaicin (5, 10, 15, 20, 25 and 30 uM) and MTT assay was performed to calculate the IC50 values. Out of 24-, 48- and 72-hours assays, due to higher experimental reproducibility of MTT assay values only 24 hours MTT assay was selected to proceed further by concluding best IC50 of Capsaicin (IC50 of 12.1uM) treatments were performed in comparison with cisplatin (control). Gene expression profiling (Caspase 3 and Caspase 8) was performed to analyze the pro-apoptotic. Significant associations with the higher relative gene fold depict the efficacy of compounds on K562 cells as shown in Figure 1.

Both biomarkers (Caspase-3 and Caspase-9) showed significant expressions as compared to cisplatin treated (control cells). Overall Relative Gene fold of Caspase-9 (13.9) was found higher as compared to Caspase-3 (12.1).

3.2. Nitric oxide (NO) releasing assay

After treating with IC50 of compounds, the NO releasing assay was performed also called Griess assay (analytical chemistry test which measures the presence of nitrite ions in the solution). Continuous rise in NO levels can be seen prominently. High NO levels confirmed the anti-proliferative potential of both compounds. The higher the NO levels, the stronger is the anti-proliferative activity as shown in Figure 2.

Addition of Capsaicin showed an abrupt rise in the graph even at low absorbance time as compared to Cisplatin (Control). Hence Capsaicin, showed the strongest growth inhibitory activity in the peak time of 100-120 min based on its optimal IC50 treatments. NO is

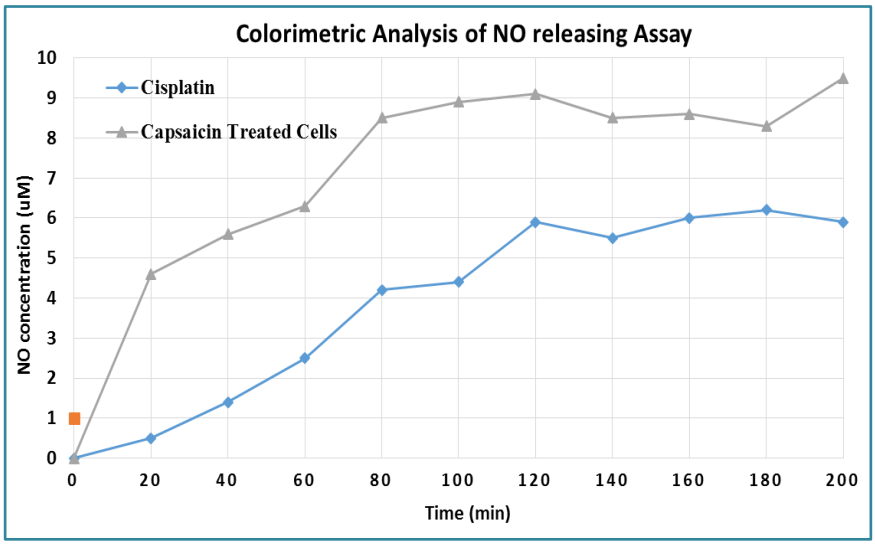

Figure 2: NO releasing assay showing anti-proliferative potential Capsaicin and Cisplatin (Control)

measured using a Griess reagent, which reacts with nitrite, a stable end product of NO, forming a colored azo compound. The intensity of the color, measured spectrophotometrically, correlates with NO levels, indicating the compounds' effect on cellular NO release, which is linked to their anti-proliferative activity.

4. DISCUSSION

Capsaicin has been shown in several cancer cell lines to have anti-oxidative, anti-inflammatory, pro-apoptotic, anti-proliferative, and anti-tumorigenic properties. It can also interfere with the beginning and progression of cancer.[14] Our study results, like those of numerous other investigations, demonstrated that treating K562 cells with capsaicin increased the expression of caspase-3 and caspase-9, confirming the compounds' pro-apoptotic (intrinsic) potential as a significant factor in the reduction of cell proliferation.[15,22]

Utilizing natural substances to improve cancer treatment has been the subject of growing recent research, especially when it comes to leukemias, such as chronic myelogenous leukemia (CML).[4] Blood cancers like leukemias are difficult to treat, since current chemotherapy methods are aggressive and can cause severe side effects and unpredictable treatment results.[16] Recent research into alternative approaches has brought to light the potential of natural phytochemicals as a supplement to traditional medicines. Promising options include phytochemicals produced from plants and phenylpropanoids, which are present in essential oils. [17] These substances target several pathways involved in tumor growth and leukemia cell-specific survival strategies, such as K562, and have shown notable anticancer effects.[18]

One substance found in chili peppers called capsaicin, for instance, has been shown to have the capacity to cause leukemia cells to undergo apoptosis via the production of reactive oxygen species (ROS) and the alteration of important communication pathways.[19] This chemical has promise in both decreasing the viability of cancer cells and increasing their sensitivity to alternative therapy.[20] Furthermore, synergistic effects have been demonstrated when natural phytochemicals are combined with well-known chemotherapeutic drugs as ciprofloxacin.[21] Capsaicin, the main ingredient in chili peppers, has been demonstrated to alter NO activity in K562 cells, a model system used in leukemia research. Studies show that treating these cells with capsaicin increases the generation of NO in these cells. For example, NO has been found to be raised in K562 cells when they are exposed to capsaicin, which may indicate that NO plays a part in the cytotoxicity caused by capsaicin.[23]

Similarly, research results showed that capsaicin increases NO generation in many kinds of cells, which may be related to its anti-tumor properties.[24] Additionally, a study showed that NO mediates the apoptotic effects of capsaicin on cancer cells, including those originating from leukemia.[25] These findings point to NO as a key signaling molecule that plays a role in capsaicin's mode of action against leukemia cells, indicating the need for more research on the potential therapeutic uses of this compound.

5. CONCLUSION

Our findings support the evidence of pro-apoptotic potential of capsaicin on the K562 cells. It was also noted that the capsaicin showed strong chemoprotective potential. The mechanism of action of these compounds, in combination, needs to be studied in vivo.

6. Future Prospects

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 can contain several anti-cancer components in it. Furthermore, the molecular mechanism of action of capsaicin with other anti-metastatic and anti-proliferative biomarkers must be studied.

7. Data availability

The numerical data generated in this study is available with the corresponding author.

8. Conflict of interest

The authors declare no conflict of interest. The study was carried out by the institutional resources of the involved institutions, and no external or industry funding was involved.

9. Authors' contribution

DD, PN, HMHK: Conception and Design of work, Acquisitions and Analysis, Agreement to be accountable for all aspects of work

MA, AM: Resulting and Interpretation; Agreement to be accountable for all aspects of work

OA, WJ: Interpretation and Drafting, Agreement to be accountable for all aspects of work

DD, WJ: Final Approval and Agreement to be accountable for all aspects of work

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