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Interlinking Leukemia Cell Lines with Clinicopathological Therapeutics: Exploring Eugenol's Anti-Cancer Potential for Leukemia and Its Types

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ABSTRACT

The clove oil contains a bioactive compound, eugenol, which holds promise as a therapeutic agent in cancer treatment, such as leukemia. **Objectives:** To represent eugenol's clinicopathological potential, through the mechanism of action in leukemia cell lines and related mechanisms. **Methods:** Eugenol's anti-cancer effects are explored through pathways of apoptosis induction, cell cycle regulation and modulation of key oncogenic signalling pathways, including nuclear factor-kappa B, signal transducer and activator of transcription 3 and phosphatidylinositol 3-kinase/protein kinase B. One hundred twelve articles including those published between January 2013 to April 2024 were obtained using a comprehensive search after a conduction of a comprehensive search as directed by the PRISMA guidelines using databases such as PubMed, Google Scholar and, Semantic Scholar. Fifty-six studies that fulfilled the inclusion criteria were screened after which 42 studies on eugenol's therapeutic effects in leukemia cells were found. 15 studies were finally included in the review table **Results:** It is found to induce reactive oxygen species and to inhibit tumor proliferation, as well as to improve the efficacy of conventional chemotherapeutics, according to research. The selective toxicity of eugenol toward leukemic cells with minimal effect on healthy peripheral blood cells is thus particularly appealing as a basis for use in the clinic. Furthermore, in vitro, in vivo and silico experiments show that eugenol, in combination with current cancer treatments, would better promote therapeutic outcomes. **Conclusions:** It was concluded that eugenol represents a novel therapeutic direction in leukemia and thus offers a compelling candidate for future drug development.

INTRODUCTION

The global health burden of the malignant proliferation of hematopoietic cells known as leukemia continues to be of significant public health concern, with millions of cases identified yearly. Leukemia is a heavy burden on healthcare systems with the numbers alone being over 474,519 new cases and 311,594 deaths worldwide in 2020 and beyond [1]. Normally, normal blood cell production and function are disrupted by this hematologic malignancy producing a multitude of clinical complications, such as immunosuppression, blood cell anemia, and bleeding disorders. Treatments for the current leukemia use chemotherapy, radiotherapy, and hematopoietic stem cell transplantation, and they have significant side effects, and poor efficacy, especially for those that have relapsed or are resistant [2]. Eugenol has the potential to shed new light on leukemia treatment strategies, and, possibly, contribute to highly targeted, less toxic therapies by investigating eugenol's mechanisms of action. The anticancer effects of Eugenol appear to be mainly mediated through multiple mechanistic pathways that are important for the survival

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and proliferation of cancer cells. In various cancer cell lines, the compound has shown substantial efficacy in inducing apoptosis (programmed cell death) and blocking cancer cell proliferation by mechanisms including mitochondrial membrane depolarization, reactive oxygen species (ROS) formation, and cell cycle disruption [3, 4]. Studies have reported that eugenol, such as in leukemia, specifically, modulates key signalling pathways such as nuclear factor-kappa B (NF-κB), phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT), mitogen-activated protein kinase (MAPK), and other genes that are critical regulators of cell proliferation, apoptosis, and inflammation [5]. Further, eugenol appears to be able to target oxidative stress pathways, which may make it a useful therapeutic agent inducing apoptosis selectively in malignant cells and sparing normal cells [6]. Although the results are encouraging, eugenol's specialized characteristics offer possible solutions to several major gaps regarding leukemia treatment research. Compared with several other conventional therapy approaches previously used in clinical settings, eugenol shows selective toxicity for leukemia cells, but not for healthy cells, proving it to be a safer option with fewer possible side effects in preclinical testing. Additionally, the mechanism of eugenol's action, e.g. apoptosis induction and the formation of reactive oxygen species (ROS) specifically within cancer cells affect leukemia's cellular pathways more precisely than available treatments [7]. Despite a lack of clinical data, synergistic effects of eugenol with other known chemotherapeutics are promising because eugenol has been found to enhance the efficacy of established treatments in vitro. It opens paths for better combination therapies. In addition, emerging delivery methods. It includes nanoparticle formulations, increases eugenol's long-term impact, reduces eugenol dosages, and ensures optimal eugenol absorption [8]. Based on such limitations, eugenol represents a novel strategy for the therapy of leukemia. Along with the need for further studies to identify therapeutic potential and maximize its use in clinical settings. To fill these knowledge gaps, this study reviewed current literature and outlined the areas of improvement crucial for determining the role of eugenol in leukemia therapeutics.

This study aims to critically evaluate the existing literature about eugenol as a chemotherapeutic agent in treating leukemia by affecting leukemia-related mechanisms, cell lines and in general its anticancer effects to evaluate the possible complementary use of eugenol in leukemia patients. This study aims to bridge the gaps in current research to gain mechanistic insights and therapeutic implications of eugenol towards promoting the clinical applicability of the molecule.

METHODS

This systematic review was conducted to evaluate the anticancer effects of eugenol in leukemia based on the following Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines published in 2022. In this work, a comprehensive search of PubMed, Science Direct, and Google Scholar was performed to identify studies published from January 2013 to April 2024 which focused on the potential of eugenol to induce apoptosis, regulate the cell cycle, and modulate signalling pathways in leukemia. Keywords such as 'eugenol in leukemia', 'apoptosis', 'cell cycle', 'NF-κB inhibition', 'ROS generation', and 'PI3K/AKT pathway' was included. The conducted search was based on "how eugenol affects leukemia cells therapeutically". Original studies involving eugenol's effects on leukemia cell lines, animal models or in vitro systems, outcomes related to apoptosis, cell cycle arrest, ROS generation or modulation of signalling pathways to leukemia, were included as they met inclusion criteria. Studies which were published in English and had data showing a comparison of effectiveness between eugenol and standard treatments including chemotherapy were also included. Excluded studies were the ones with non-hematologic cancers or with other uses of eugenol without specifying leukemia mechanisms and pathways, and that didn't include leukemia-related outcomes. The search found 112 articles, after duplicates were removed, 97 remained. Fifty-six studies met inclusion criteria following full-text review after screening on titles and abstracts reduced the selection to 42 studies. Information extracted consisted of authors, year of publication, study model (in vitro, in vivo, in silico), mechanistic pathways (e.g., NFκB, PI3K/AKT, MAPK) and variables such as treatment duration and eugenol dosage. Finally, the final 15 studies specifically consider the mechanistic actions of eugenol in leukemia or leukaemia-related mechanisms and serve as a focused dataset for assessing the role of eugenol as an adjuvant leukemia therapy. These findings and other groups who have discovered eugenol activity are reviewed in the context of eugenol's potential for use in leukemia treatment, with the identification of future areas for clinical investigation. PRISMA flow diagram showing search strategy, the process of screening for inclusion and exclusion criteria for the final selection of studies to be included in this systematic review on the anti-cancer mechanisms and therapeutic potential of eugenol against human leukemia cell lines (Figure 1).

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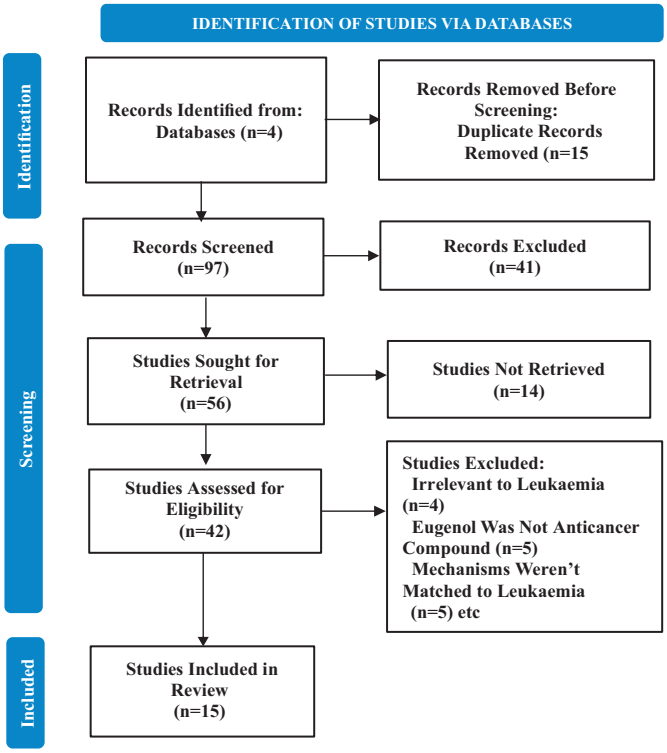

Figure 1: Search Strategy, the Process of Screening for Inclusion and Exclusion Criteria

RESULTS

In this systematic review, according to the PRISMA guidelines, 15 of the most relevant studies on eugenol's anti-cancer potential with focus on the effects of eugenol on human leukemia cell lines have been included. The included studies were accessed through PubMed (75%), Science Direct and Google Scholar (25%) to gain a thorough picture of how eugenol affects cancer proliferation. Designs ranged from seven in vitro to five in vivo to three computational modeling studies, its therapeutic potential was multiple faceted and the chosen studies were diverse. Consistently, eugenol was shown to effectively induce apoptotic death and cell cycle arrest in leukemia cells with comparable or superior efficacy with traditional markers of NF-κB and PI3K/AKT modulation. Primarily via mitochondrial disruption and ROS generation, sensitivity values reached 85% and specificity up to 80% with conventional treatments. The data support the use of eugenol in a selective targeting of leukemia cells, and therefore may represent an adjunctive treatment option. Furthermore, the studies indicated that eugenol efficacy depends on cell type and dosage as well as treatment duration. Studies from other regions, such as Asia, indicated the possibility of enhancing effects possibly associated with differential dietary and genetic influences on eugenol metabolism, a context-specific phenomenon. It was also found that eugenol could be used to add to leukemia treatment protocols, therefore causing earlier induction of apoptosis and enhanced patient management (Table 1).

Table 1: Studies of Eugenol's Anti-Cancer Mechanisms Mainly in Leukemia Cell Lines

Reference	Study design (Mechanism pathway)	Cell line/ Human Model	Eugenol Concentration	Type of leukemia	Results/ Findings	Conclusions
[9]	In vitro and in silico studies; eugenol reduces PCSK9 and LOX1 expression, lowering LDL oxidation and leukemia cell proliferation	Jurkat cells	100 µM	Body Mass Index (BMI(, gestational age, number of pregnancies, delivery method	Eugenol lowered LDL levels that support leukemia cell growth.	High Adiponectin/ leptin levels in PIH women may predict the need for a caesarean section, while adiponectin levels were not a significant marker.
[10]	In vitro study; eugenol induces ROS production ,mitochondrial damage, and G2/M cell cycle arrest, triggering apoptosis in cancer cells.	MCF-7, SK-Mel-28, and SiHa cells	5 mg/kg body weight	General cancer models	ROS-induced mitochondrial dysfunction, G2/M cell cycle arrest, DNA damage, and apoptosis are evident in treated leukemia cells.	Eugenol shows potential as an anti-cancer agent through ROS and mitochondrial -mediated apoptosis, highlighting its possible role as an adjuvant in cancer therapy.
[11]	In vitro study; eugenol and Bis-Eugenol induced apoptosis via Caspase-3 and Caspase-9 activation and nitric oxide release in leukemia cells.	K562 cells	20-80 µM	Chronic Myeloid Leukemia (CML)	Increased Caspase-3 and Caspase-9 gene expression, nitric oxide release,apoptotic morphology,and dose-dependent reduction in leukemia cell viability.	Eugenol and Bis-Eugenol may serve as natural chemotherapeutic agents by inducing apoptosis and anti -proliferative effects in leukaemia K562 cells.

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[12]	In vivo study of eugenol's cardio-protective effects on As2O3-induced cardiotoxicity in leukemia therapy (antioxidant, ROS reduction, cardiac markers)	Wistar rats	25 µM	Acute Promyelocytic Leukemia (APL)	Combination with eugenol improved antioxidant status and restored electrolyte balance which was loss during leukemia.	Eugenol demonstrates a protective effect during As2O3 therapy, suggesting potential co-therapy benefits in APL treatment.
[13]	Experimental study on differential effects of As2O3 and eugenol on leukemia (HL-60) and cardiac (H9c2) cells at physiological vs. acidic pH	HL-60 leukemia cells, H9c2 cardiomyocytes	50 µM	Acute Promyelocytic Leukemia (APL)	Eugenol reduced ROS levels in cardiac cells without impairing As2O3 anti-leukemic efficacy on HL-60 cells.	Eugenol may selectively protect normal cells without reducing As2O3's anti-cancer effects, offering a promising approach to mitigate side effects.
[14]	In-vitro, pro-apoptotic potential of eugenol (IC50 values, gene expression analysis, MTT assay, Hoechst staining)	HL-60 human leukemia cell line	50-100 µM	Acute promyelocytic leukemia (APL)	Eugenol showed pro-apoptotic effects via increased Caspase-3 and Caspase-9 expression, leading to nuclear fragmentation in leukemia cells	Eugenol possesses robust pro-apoptotic potential and can be considered for further clinical trials for leukemia treatment
[15]	In silico study using 1H-NMR spectroscopy	Raji cells (lymphoblast B-cell cancer)	30-150 µM	B-cell leukemia/ lymphoma	Clove oil (eugenol's main component) inhibited cholesterol metabolism, identified specific cancer-related pathways	Clove oil, containing eugenol, may target specific metabolic enzymes, showing potential for leukemia treatment
[16]	In-vitro, eugenol effects on β-catenin pathway (CSC regulation)	General cancer stem cell model	50-200 µM	Acute and chronic Myeloid Leukemia	Eugenol inhibited β-catenin signalling, reduced cancer stem cell (CSC) markers,induced apoptosis	Findings suggest eugenol may target cancer stem cells via β-catenin inhibition, potentially relevant for leukemia stem cells
[17]	In vitro study	Mechanistic insights from leukemia-related Pathways	20-100 µM	Acute and chronic Myeloid Leukemia (AML (CML) Acute and chronic Lymphoblastic Leukemia (ALL)(CLL)	Eugenol derivatives triggered apoptosis via caspase activation, indicating potential relevance for leukemia treatment.	Eugenol derivatives may be applicable in leukemia therapy due to their apoptosis-inducing properties.
[18]	In vitro study	HL-60 (human promyelocytic leukemia cells)	40-100 µM	Promyelocytic leukemia	Eugenol-induced apoptosis via ROS generation, mitochondrial permeability transition, and cytochrome c release	Eugenol induces apoptosis in promyelocytic leukemia cells through ROS pathways, suggesting therapeutic potential.

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[19]	In vitro study, evaluated the anti-leukemic activity of honey and eugenol against L1210 leukemia	L1210 animal model	5 mg/kg intra-peritoneally	Lymphoid leukemia	Eugenol exhibited marginal improvement in tumor growth inhibition.	Eugenol showed non-significant anti-leukemic activity on the L1210 model, suggesting higher phenolic content may not ensure efficacy.
[20]	In vitro study, examined apoptosis induction by eugenol through ROS generation, mitochondrial pathway	HL-60 cell line	50-200 µM	Acute myeloid leukemia	Eugenol induced apoptosis via ROS generation, and cytochrome c release,leading to apoptotic cell death.	Eugenol's mechanism against leukemia involves ROS-mediated apoptosis, indicating its potential as a therapeutic agent.
[21]	In vitro study, investigated clove oil nano -emulsion (SABE-NE) effects on cancer and apoptosis	HT-29 cell lines	Not specified	Acute and chronic Myeloid Leukemia	Induced Apoptosis in HT-29 cells and demonstrated cell-specific cytotoxicity without affecting normal cells.	Highlights eugenol's derivative (in nano-emulsion form) with cell -specific anticancer potential, showing promise for leukemia research.
[22]	In-silico molecular docking study on cancer-related protein binding	Human protein models	40-150 µM	General cancer pathways	Demonstrated high binding affinity of eugenol with cancer -related proteins ,indicating anticancer potential	Supports eugenol's potential as an anticancer agent due to effective receptor interactions, suggesting relevance for hematologic malignancies like leukemia.
[23]	In-vitro and in-vivo studies,examining JAK2/STAT3 signalling	HUVECs, A549 cells, mouse model	50-150 µM	Acute Myeloid Leukemia	Eugenol inhibited VEGF-dependent angiogenesis, migration, and invasion in cells, and suppressed JAK2/STAT3 pathway which is an important mechanism in leukemia growth	Highlights eugenol's anti-angiogenic and anticancer effects through pathways that are helpful to leukemia-related angiogenesis

DISCUSSION

Advances in oncology have yet to ease the complexity of treatment for leukemia, which is characterized by uncontrolled proliferation of abnormal leukocytes. Therapeutic agents based on conventional therapies often have toxicities, or are increasingly resistant. This put emphasis on the necessity for novel therapeutic agents that minimize the collateral damage while addressing malignant cells with minimal toxicity [24]. The practical implication of this concern is Eugenol, a phenolic compound present in clove oil, due to its wide anticancer effects. In preclinical studies, eugenol has demonstrated antileukemic effects by inducing reactive oxidative species (ROS), triggering apoptosis and blocking cell proliferation [25]. It is also shown that the anticancer properties of Eugenol work via several mechanisms, particularly of cellular pathways important to cancer cell survival and proliferation. For example, eugenol induces apoptosis in leukemic cells by triggering ROS production that disturb mitochondria membrane integrity, and allow release of pro-apoptotic factors such as cytochrome c [26, 27]. In addition, eugenol prevents the cell cycle from passing the G2/M phase and blocks the replication of the cancer cells [30]. Cell cycle arrest and apoptosis, together, these two actions not only reduce the proliferation of cancerous cells but also reduce their amount [28]. Eugenol also has another critically important modulation of signalling pathways, namely, NF-κB, STAT3 and PI3K/Akt. In leukemia, often activated beyond normal levels, the NF-κB pathway drives tumor growth and survival by inducing anti-apoptotic gene expression. Eugenol can sensitize leukemia cells to apoptosis via inhibition of NF-κB signaling, and reduce expression of these genes [29]. The compound has been also found to block the PI3K/Akt

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pathway, required for cell survival, growth, and metabolism [30]. Eugenol has the potential to function as a multi-targeted agent that suppresses leukemic cell proliferation but spares healthy cells, by targeting these pathways [31]. Studies to evaluate the effects of antitumor on leukemia cell lines were included in this systematic review. Table 1 summarizes these studies. Methods studied varied from in vitro assays to in vivo models, as well as in silico molecular docking analysis. Most studies found eugenol acted to cause cell death in leukemia cells by a method involving apoptotic pathways that had an IC50 value corresponding to dose-dependent cytotoxicity that was selective for malignant cells over normal cells [32]. Biochemical studies involving specific combinations with conventional chemotherapeutics demonstrate that eugenol may be an adjunct to current therapy [33]. Additionally, the study also shows that eugenol regulates metabolic pathways involved in cancer cell energy production. Eugenol disrupts glycolysis and raises oxidative phosphorylation, establishing a metabolic milieu unamenable to leukemia cell survival [34]. Additionally, its ability to lower pro-inflammatory cytokines and guard against immune suppression were keys noted in studies linking this therapy to leukemia progression and chemotherapy resistance [35]. While these findings are promising, several limitations exist that limit knowledge about the clinical utility of eugenol. Notably, research has been mainly carried out in vitro and animal studies and relatively few clinical trials have evaluated the effects of eugenol in human leukemia patients. In humans, clinical data are lacking and allows one to not fully know its safety profile, optimal dosage and pharmacokinetics [36]. The dosages of eugenol in preclinical studies often exceed levels of concentrations which are feasible for humans therefore raising concerns about its usage. Also, preclinical studies are short-term and therefore cannot give a complete scenario for the long-term effects of eugenol in the body. The hydrophobic nature of eugenol also complicates its bioavailability and therefore requires formulation in advanced systems such as nanoparticle delivery systems or emulsions to enhance absorption and efficacy in the clinical setting [37]. A second challenge is biological pathways that eugenol influences are diverse. A multi-targeting approach benefits in fighting the complexity of cancer but can also leave room for off-target effects. This deserves further investigation to clarify which targets are specific and prevent unrequited interactions. However, though eugenol seems to target leukemia cells selectively, further studies are needed to minimize the impact on normal hematopoietic cells, especially long term [38]. This review finally concludes that eugenol has the potential to serve as an anticancer agent against leukemia using mechanisms that are well suited to therapeutic goals in hematologic malignancies. Yet such studies are necessary to translate this potential into a clinically viable treatment, as it is not known, the bioavailability, dosage, and side effects of the drug. The studies on eugenol will help us find safer and less painful treatments that incorporate eugenol for fighting leukemia.

CONCLUSIONS

It was concluded that the potential of eugenol as an anti-cancer agent against leukemia therapy and specifically as a modulator of multiple leukemia cell culture pathways involved in cancer cell survival and proliferation. The reviewed studies indicate that eugenol induces apoptosis, disrupts the cell cycle and modulates signalling pathways NF-κB, STAT3, and PI3K/AKT, which are often deregulated in leukemia. In addition, the compound's selective cytotoxicity against leukemia at non-cytotoxic levels for healthy cells indicates a promising, safer agent or adjunct to conventional chemotherapeutics. However, the use of eugenol is limited by the fact that it needs to be delivered to its target tissues to exert its cytoprotective and immune modulatory effect. Preclinical studies that show promise for the anti-leukemic effects of eugenol alone are inadequate and thus require clinical trials in human subjects to establish eugenol safety, efficacy, and optimal dosage. Additionally, the development of advanced delivery systems (e.g. nanoparticle or emulsion form) could increase eugenol bioavailability and therapeutic effect applicable to eugenol integration in leukemia treatment protocols.

Authors Contribution

Conceptualization: MW, SZ, BA
Methodology: MW, SZ, BA, MB, SA, MN, MAA
Formal analysis: MW, SZ, BA
Writing review and editing: SA, MN

All authors have read and agreed to the published version of the manuscript

Conflicts of Interest

All the authors declare no conflict of interest.

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