Comparative Evaluation of 1,8 cineole on inhibition and migration of Human Ovarian Cancer Cell Lines SKOV3 and PA-1

Saloni Joshi[1], Ruby Beniwal[2], Reema Gabrani [1*]

[1]Department of Biotechnology, Jaypee Institute of Information Technology, Sector-62, Noida,

Uttar Pradesh, India 201309

Corresponding Author Email: reema.gabrani@mail.jiit.ac.in

Abstracts

Ovarian cancer is a leading cause of mortality among gynecological malignancies, primarily due to delayed diagnosis and the limited effectiveness of existing therapeutic strategies. The search for safer and more effective treatment options has led to increasing interest in plant-derived phytocompounds, which exhibit diverse biological activities with comparatively fewer adverse effects. This study evaluates and compares the effects of the phytocompound 1,8-cineole on human ovarian cancer cell lines SKOV3 and PA-1. The anti-cancer activity of 1,8 cineole was evaluated using in vitro cell viability assays, supported by cell migration-based assays to assess changes in cellular behavior following treatment. The results have reported a significant level in reduction of growth and migratory potential upon 1,8 cineole exposure, indicating their ability to interfere with key processes involved in cancer progression

Keywords: Anti-proliferative, Cell migration, Ovarian cancer, PA-1, Phytocompounds, SKOV3

1.0 Introduction

Ovarian cancer (OC) ranks as the eighth most frequently diagnosed malignancy in women globally and represents the deadliest form of gynecological cancer. High mortality rates are primarily attributed to asymptomatic early stages, advanced disease at diagnosis, chemoresistance, and tumor recurrence. Despite advances in targeted therapy and platinum-based chemotherapy, long-term survival remains limited (Sung et al., 2021), (Wang and Yu, 2025), (Torre et al., 2018).

Emerging evidence highlights the potential of plant-derived bioactive compounds as adjunct or alternative therapeutic agents due to their multitargeted mechanisms and relatively lower systemic toxicity. Monoterpenes, particularly those present in essential oils, have shown promising anticancer activity.

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1,8-Cineole (eucalyptol) is a cyclic monoterpene oxide commonly isolated from *Eucalyptus globulus*, rosemary, sage, and other aromatic plants. It is widely found to be used in pharmaceutical and cosmetic industries for its anti-inflammatory and antimicrobial properties. Recent studies suggest that 1,8-cineole also exhibits anticancer potential through modulation of oxidative stress, apoptosis, cell cycle regulation, and inflammatory pathways (Bakkali et al., 2008), (J et al., 2003).

SKOV3 and PA-1 ovarian cancer cell lines model different therapeutic responses and are often used together to assess activity against both resistant and sensitive disease. SKOV3 is an epithelial ovarian cancer line lacking functional p53, leading to impaired apoptosis and resistance to platinum drugs through activation of survival pathways like PI3K/Akt and NF-κB. In contrast, PA-1 retains wild-type p53, allowing efficient cell-cycle arrest and apoptosis and is therefore considered chemosensitive. Hence, compounds effective in both cell lines may overcome chemoresistance. Hence, the present study investigates the antiproliferative and anti-migratory potential of 1,8-cineole in SKOV3 and PA-1 ovarian cancer cell lines. (Domcke et al., 2013), (Beaufort et al., 2014).

2. Materials and Methods

2.1 Cell Culture

The human ovarian cancer cell lines SKOV3 and PA-1 cells were maintained in RPMI medium supplemented with 10% fetal bovine serum, 0.1% antibiotics, and incubated under standard culture conditions (37 °C, 5% CO_2).

2.2 Compound Preparation

1, 8 cineole was prepared by dissolving approximately 16 µL of analytical-grade 1,8-cineole in DMSO to obtain a 100 mM stock solution, which was subsequently diluted in culture medium ensuring the final DMSO content remained below 0.1%.

2.3 Cell Viability Assay (MTT)

Cell viability was measured by MTT assay, which estimates metabolic activity of living cells through mitochondrial reduction of MTT to formazan. SKOV3 and PA-1 (1×10^4 cells/well) were seeded in 96-well plates and incubated for 24 h for attachment. Cells were then exposed to varying concentrations of 1,8-cineole for another 24 h. Afterwards, 20 µL MTT solution (5 mg/mL) was added, and plates were incubated at 37 °C for 4 h. subsequent, DMSO was added and absorbance was measured at 570 nm using a microplate reader (Hasan et al., 2023), (Xiong et al., 2021).

2.4 Wound Healing Assay

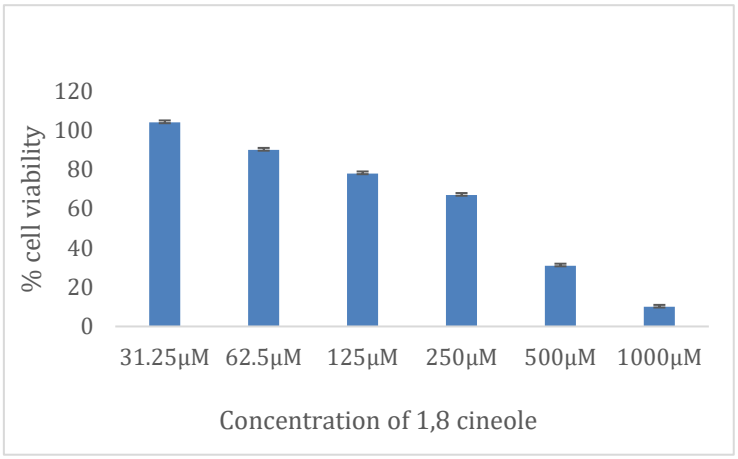
SKOV3 and PA-1 (1×10^5 cells/well) were seeded in 6-well plates for 24 h, until a confluent monolayer was formed. A scratch was made using a sterile pipette tip, debris were removed with PBS, and cells were treated with the IC_{50} concentration of 1,8-cineole. Migration into the wound area was monitored at 0 and 24 h using an inverted microscope (Wang et al., 2020), (Vijayaraghavan et al., 2024), (Gautam et al., 2023).

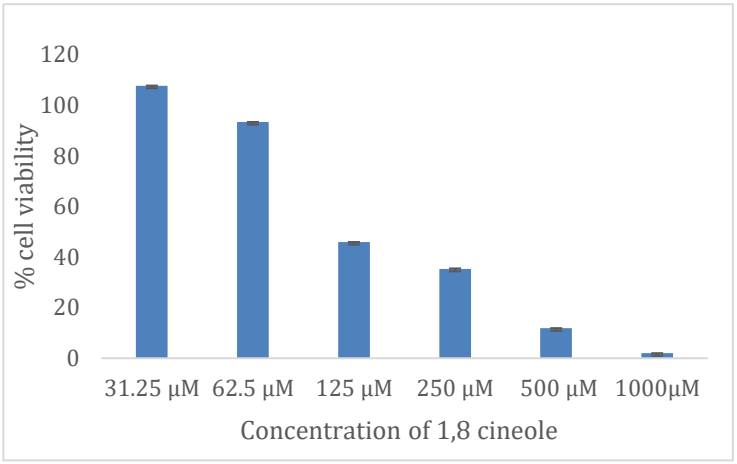
3.0 Results and discussion

3.1 MTT assay results

The inhibitory potential of 1,8-cineole in SKOV3 and PA-1 ovarian cancer cells was assessed using the MTT assay. A dose-dependent decline in cell viability was observed in both cell lines following treatment. In SKOV3 cells, viability decreased progressively from, indicating strong growth inhibition with IC_{-50} of 289µM (Figure 1). A similar declining trend was also observed in PA-1 cells, with IC_{-50} of 179µM (Figure 1), confirming consistent cell inhibitory activity across ovarian cancer cells. Overall, the findings indicate that 1,8-cineole reduced ovarian cancer cell viability in a concentration-dependent manner.

The concentration-dependent decrease in viability in SKOV3 and PA-1 cells indicates that 1,8-cineole exhibited inhibitory activity on cancer cells. Monoterpenes including cineole are known to inhibit the PI3K/Akt pathway, leading to reduced cyclin-D1 mediated cell-cycle progression and growth arrest (Gao et al., 2022) In parallel, cineole promotes mitochondrial apoptosis by increasing the ratio of Bax/Bcl-2, triggering the release of cytochrome-c and caspase-3 activation, while suppression of NF-κB signaling decreases survival gene expression such as Bcl-2 and survivin (Rodenak-Kladniew et al., 2020). Additionally, modulation of ROS-dependent MAPK/ERK signaling contributes to reduced proliferation. The lower IC_{50} in PA-1 cells likely reflects differences in basal Akt and p53 signaling sensitivity (Greiner et al., 2013). Overall, the data suggests that 1,8-cineole inhibited ovarian cancer cell viability through combined PI3K/Akt suppression and mitochondria-mediated apoptosis(Jian et al., 2017), (Machado et al., 2022), (Wu et al., 2025), (Cha et al., 2010).





A B

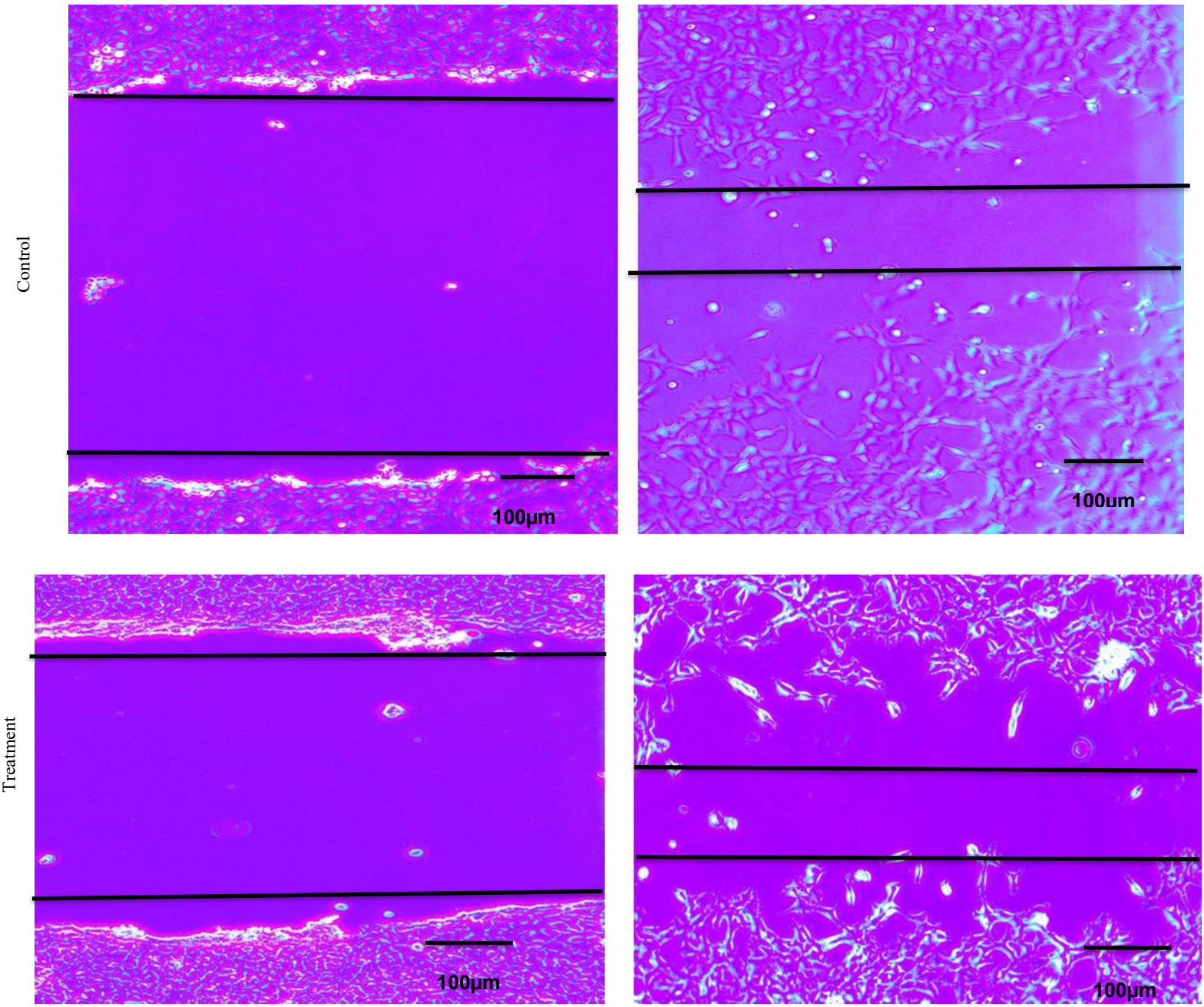
Figure 1: The inhibitory effects of 1,8 cineole on ovarian cancer cell lines (A): SKOV3 cells, (B) PA-1 cells

Cell migration wound healing assay was performed on SKOV3 and PA-1 ovarian cancer cells. Untreated control cells showed rapid wound closure over 24 h with 90% gap closure whereas 1,8 cineole treated showed 40% wound closure in PA-1 cells (Figure 2). In SKOV3 cells, the wound gap was completely closed (99%) in untreated control SKOV3 ells after 24 h, whereas treatment with 1,8-cineole resulted in only 46% closure, indicating marked inhibition of cell migration (Figure 2).

The wound-healing assay demonstrated that 1,8-cineole markedly suppressed ovarian cancer cell motility, reducing gap closure from ~90–99% in controls to ~40–46 % after 24 h in both PA-1 and SKOV3 cells. Cancer cell migration is promoted by epithelial–mesenchymal transition (EMT), cytoskeletal reorganization, and degradation of the extracellular matrix, which are primarily controlled by the PI3K/Akt, NF-κB, and MAPK/ERK signaling pathways (Lamouille et al., 2014).

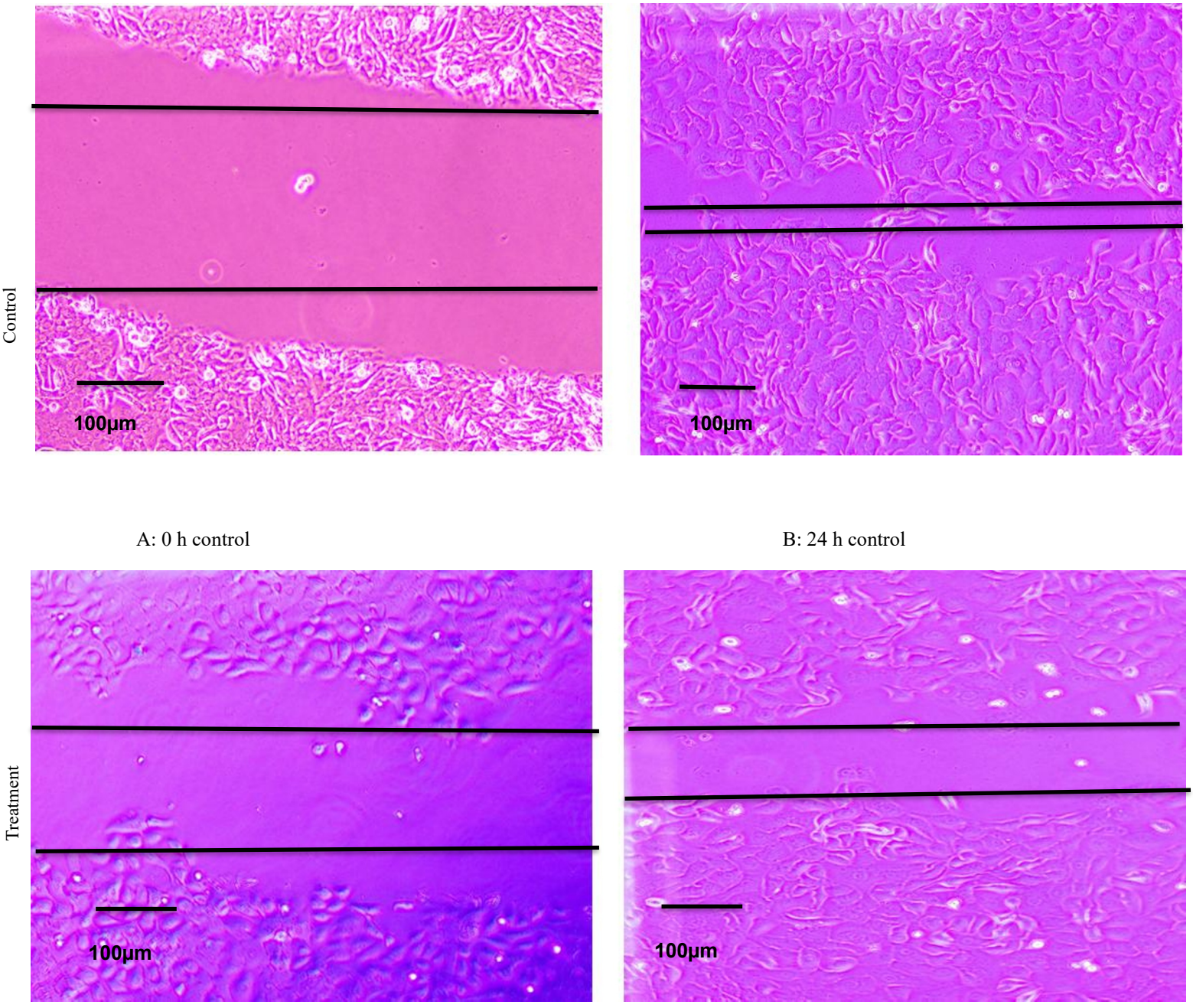
Previous mechanistic studies have shown that cineole inhibited Akt phosphorylation, stabilizes E-cadherin, and prevents β-catenin nuclear translocation, thereby suppressing EMT-associated transcription factors such as Snail and Twist and limiting motility. In addition, cineole downregulated NF-κB–dependent expression of matrix metalloproteinases (MMP-2 and MMP-9), reducing extracellular matrix breakdown required for wound closure and metastatic spread. Suppression of ERK signaling further interfered with actin polymerization and lamellipodia formation, impairing directional migration. Collectively, the reduced gap closure observed in both cell lines suggests that 1,8-cineole inhibited ovarian cancer migration through coordinated modulation of EMT, proteolytic activity, and

cytoskeletal dynamics rather than simple cytotoxicity (Takeda et al., 2022), (Moteki et al., 2002), (Samson et al., 2022).



C: 0 h D:24 h

Panel II: SKOV3



A: 0 h control

B: 24 h control

Figure 3. Comparative evaluation of wound-healing ability in PA-1 (panel I) and SKOV3 (panel II

For both panels, **(**A) 0 h control, (B) 24 h control, (C) 0 h after 1,8-cineole treatment, (D) 24 h after 1,8 cineole treatment.

4.0 Conclusion

This study demonstrates that 1,8-cineole exerted significant anticancer activity in ovarian cancer models, producing dose-dependent growth inhibition and marked suppression of cell migration. Importantly, the compound was effective in both PA-1 (chemosensitive, p53-competent) and SKOV3 (chemoresistant, p53-deficient) cell lines, indicating inhibitory activity independent of classical apoptosis competence and suggesting the ability to overcome resistance mechanisms.

Abbreviations

AKT -Protein Kinase B (PKB)

Bax - Bcl-2–associated X protein

Bcl-2- B-cell lymphoma 2

EMT- Epithelial–Mesenchymal Transition

ERK - Extracellular Signal-Regulated Kinase

MAPK - Mitogen-Activated Protein Kinase

MMP-2 - Matrix Metalloproteinase-2

MMP-9 - Matrix Metalloproteinase-9

MTT -3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

NF-κB- Nuclear Factor kappa-light-chain-enhancer of activated B cells

p53 -Tumor Protein 53

PI3K - Phosphoinositide 3-Kinase

ROS - Reactive Oxygen Species

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Credit authorship contribution statement

Saloni Joshi: Writing, editing of original drafts

Reema Gabrani: Editing, reviewing, supervision, and validation of the original draft

Ruby Beniwal: Editing, reviewing, and supervision.

Declaration of Competing Interest

The authors declare no competing financial or non-financial interests that could have influenced the work reported in this review article.

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Declaration of Generative AI and AI-assisted technologies in the writing process

The author used AI tools to correct grammar and improve the quality of writing and paraphrasing and enhance the quality of images.

Data availability

No data was used for the research described in the review.

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