Evaluation of the Anti-Cancer Potential of Sulforaphane-Loaded Small Extracellular Vesicles Derived from Human Menstrual Blood Mesenchymal Stem Cells

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ABSTRACT:

Lung cancer is the leading cause of cancer related deaths and accounts for more deaths than colon, breast or prostate cancer while being the second most common type of cancer in both males and females worldwide. Traditionally, treatment for lung cancer includes surgery, chemotherapy and radiation. However, these treatments have shown little therapeutic success and adverse effects like drug resistance. Due to this the need for alternative treatment options has been a topic of interest and one of the solutions is cell free therapy which is particularly a new modality in this field. Secretory factors that are found in Mesenchymal Stem Cells (MSCs) which include Extracellular Vesicles (EVs), chemokines, cytokines and other metabolites have shown to possess anti-tumor effects. Sulphoraphane (SFN), an active compound found in cruciferous vegetables like broccoli and cauliflower, has had several studies for its antioxidant, anti-inflammatory and anticancer effects. This study focuses on priming of Human Menstrual Blood MSCs (MB-MSC) derived small Extracellular Vesicles (sEVs), with SFN as an alternative option for lung cancer treatment. The anticancer activity of the SFN loaded sEVs was assessed by in vitro studies on A549 lung cancer cell line. This study establishes a natural, low-toxicity drug carrier capable of efficient therapeutic delivery to cancer cells and also showed that SFN-primed sEVs significantly reduced cell proliferation in vitro, validating their potential as a promising cell-free therapy for lung cancer.

Keywords: Lung cancer, Human derived mesenchymal stem cells, small Extracellular Vesicles, Sulphoraphane, Anti-cancer.

1. **INTRODUCTION**:

Cancer is the second most common cause of death in the United States with the deadliest cancer types being lung, colon, pancreatic and liver with lung cancer leading in mortality with 125,070 deaths, followed by colon cancer with 53,010 deaths in the United States in 2024 alone [1]. Lung cancer is divided into groups based on histology and tumor morphology. Small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) are the two primary forms. Adenocarcinoma (LUAD), squamous cell carcinoma, and giant cell carcinoma are other subtypes of NSCLC. 85% of cases of lung cancer are caused by NSCLC. [2]. The pathogenesis of each type of lung cancer is extensive and varies but they all involve the activation of a pathway that promotes tumor growth and inhibition of the tumor suppressor pathways. The common gene mutations involved in the pathogenesis of lung adenocarcinoma is given in figure 1 [3].

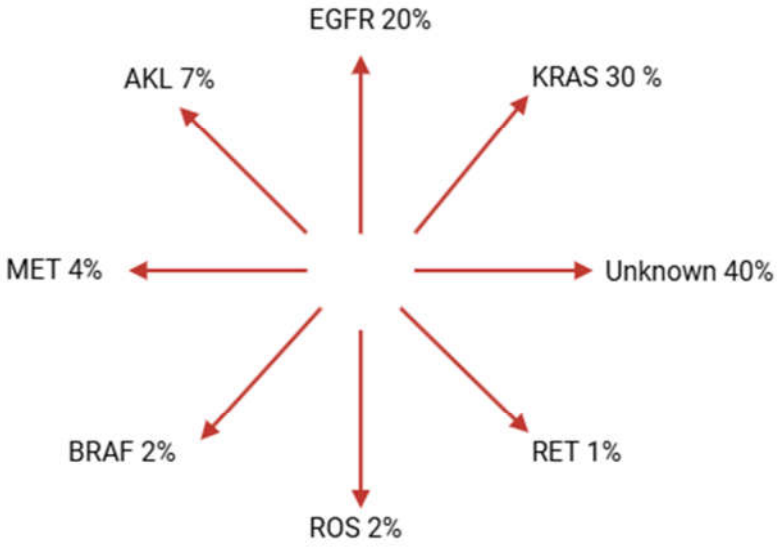

Figure 1. Common mutations in lung adenocarcinomas. Created in https://BioRender.com

Even though several treatment options are available, the eradication of this life threatening condition is still not available and in the last decades, little significant clinical improvements in the outcome of lung cancer have been achieved [4]. The current conventional treatments like chemotherapy, radiotherapy, surgery etc. possess limitations like drug solubility, drug resistance, adverse reactions etc.

In order to get around these restrictions, developments in cell therapy and cell-free therapy during the past few decades have shown promise in treating a number of illnesses, including cancer immunotherapy, diabetes, neurological diseases, and arthritis. [5]. The human umbilical cord derived mesenchymal stem cells (hUCT-MSCs) was one of the first sources of stem cells used for treatment because of its painless ease of collection and remains the most common source of stem cells to date [6,7]. Several other sources have been identified including bone marrow, umbilical cord blood, placenta and adipose tissue[8], dental pulp[9], fetal liver tissue, and lung tissue. Menstrual blood-derived mesenchymal stem cells (MB-MSCs), a unique source of MSCs, have also demonstrated promising therapeutic effects in a variety of disorders because of their many benefits, including frequent donation, numerous supplies, and periodic and non-invasive sample collection. [10,11].

MSCs have the ability to specifically target tumors and their metastases and have been employed to deliver cytotoxic effects directly into tumors. MSCs have the ability to trigger apoptosis and promote the death of lung cancer cells through the expression of Tumor Necrosis Factor (TNF)-related apoptosis-inducing ligand (TRAIL). By attaching to cell-surface death receptors, TRAIL triggers external apoptotic pathways and specifically causes cancer cells to undergo apoptosis while sparing healthy cells. Phases 1-2 of many stem cell-based clinical studies are being conducted to treat lung cancer.[12]The clinical efficacy for cell therapy-based treatment is still in the experimental phases due to MSCs characteristics of tumorigenicity, immunogenicity, and heterogeneity which can be a double edged sword for the human body [5].

Targeted in vivo therapeutic applications are made possible by a variety of synthetic nanoparticles and biological vesicles with biological selectivity found in nanotechnology-based drug delivery systems. [13]The slow-release features of nanoparticles enable continuous high local drug concentrations and nucleic acids at the tumor site, thereby enhancing anticancer efficacy which can effectively reduce their side effects and improve therapeutic efficacy [14,15]. Biological vesicles are naturally derived from bacteria, erythrocytes, or mammalian cells and MSCs which secrete extracellular vesicles [16]. Extra-cellular vehicles (EVs) include exosomes, small Extracellular Vesicles (sEVs), micro vesicles, and apoptotic bodies which are cell-secreted nanoparticles composed of a bilayer lipid membrane structure [17]. Small Extracellular vesicles are bio-derived nanocarriers typically ranging from 30 to 150 nm in size. They exhibit excellent biocompatibility, structural stability, and relatively low immunogenicity [15]. Evidence shows that sEVs by itself have the ability to inhibit tumor growth, proliferation, and metastasis. The effectiveness of sEVs can be enhanced by modifying them—such as by incorporating therapeutic agents, bioengineered molecules like chemotherapeutic drugs and nucleic

acids, or by pre-treating them with phytochemicals known for their anti-cancer properties [18].

A key limitation of existing cancer therapies is the rising resistance to drugs and the unpredictable molecular responses they often produce. Medicinal plants contain phytochemicals and their derivatives, which show promise for increasing cancer patients' treatment efficacy and reducing the previously listed drawbacks.[19] Multiple studies using cell lines and animal models have demonstrated the potential of phytochemicals in both cancer prevention and therapy, with highly encouraging outcomes. These phytochemicals have been shown to reduce cell proliferation, cause apoptosis, delay metastasis, and inhibit angiogenesis [20].

Sulphoraphane (SFN) is a compound within the isothiocyanate (ITC) group of organosulfur compounds derived from cruciferous vegetables such as broccoli and cauliflower[21](Refer to figure 2). It possesses anti-inflammatory, cardioprotective, antioxidant, cytoprotective, DNA-protective, and antimicrobial activities. Furthermore, it serves as a powerful immune enhancer and detoxifying agent. SFN has been shown to demonstrate the ability to suppress and potentially reverse carcinogenesis, as well as induce apoptosis in cancer cells[22].

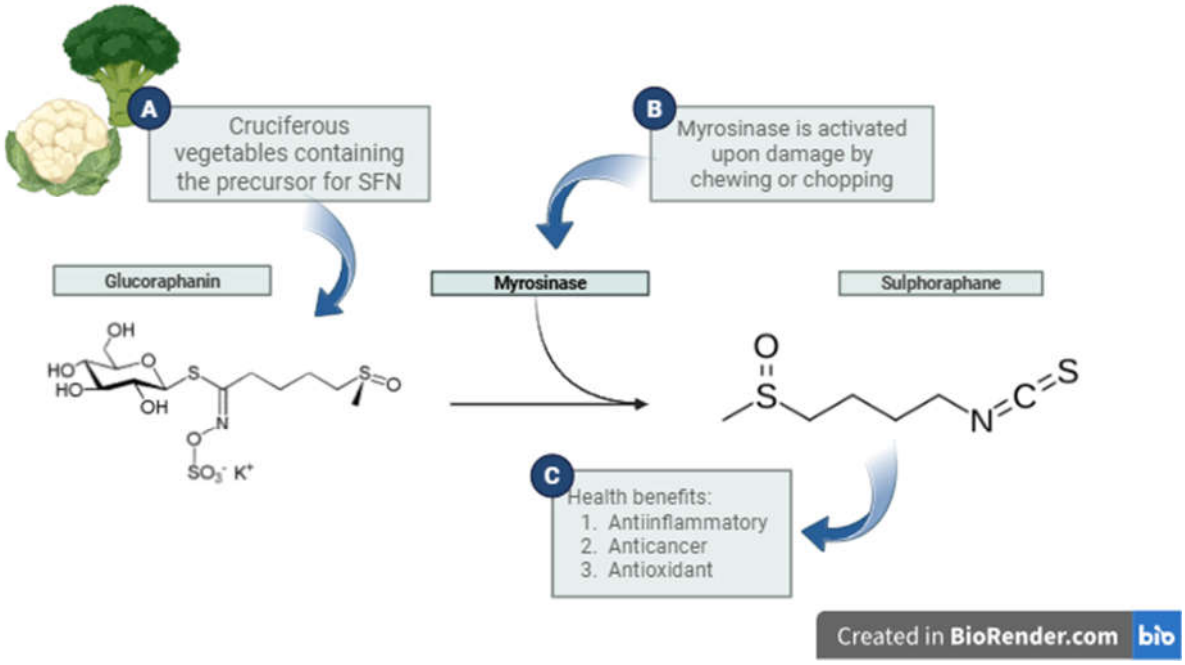

Figure 2. (A) Cruciferous vegetables especially broccoli sprouts are a rich source of glucoraphanin. (B) Glucoraphanin is converted into SFN ($C_6H_{11}NOS_2$) through the action of the enzyme Myrosinase activated by chewing or chopping. (C) SFN is shown to exhibit anticancer,

neuroprotective and anti-inflammatory properties. (Chemical structures of SFN and Glucoraphanin were sourced from their respective Wikipedia pages: https://en.wikipedia.org/wiki/Sulforaphane and https://en.wikipedia.org/wiki/Glucoraphanin.) Created using https://BioRender.com

Building on this context, our research explores a novel therapeutic strategy that combines mesenchymal stem cells-derived Small Extracellular Vesicles (MSC-sEVs) with Sulphoraphane to overcome the current limitations in cancer treatment. Specifically, we utilize MSCs isolated from Human Menstrual Blood as a bio-source for sEVs production, aiming to enhance the delivery and efficacy of the phytochemical agent.

2. MATERIALS AND METHODS

2.1. ETHICAL APPROVAL:

This study protocol was conducted with the approval of the Institutional Ethical Committee (IEC) (NO. IEC/C-P/3/2025) and lies in compliance with the Helsinki Declaration. The blood was collected with informed consent from the donor and approved by the institutional review board.

2.2.MATERIALS:

The experiments were conducted under Class 10,000 cleanroom conditions. Key components used in this study included menstrual blood (MB), small Extracellular Vesicles (sEVs) derived from MB-MSCs, and D,L-Sulforaphane (≥90% purity, HPLC; Sigma-Aldrich, Cat. No. S4441, St. Louis, MO, USA). Cell cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. Characterization on MSCs was done using flow cytometry, gene expression and karyotyping. Characterization of the sEVs was performed using nanoparticle tracking analysis (NTA). For the in vitro experiments, A549 lung cancer cell line was employed to evaluate cytotoxicity and to carry out scratch assays, evaluating the anticancer potential of SFN-primed sEVs.

2.3.Methods

2.3.1. Isolation, Culturing and Characterization of MB-MSCs

The research commenced with the collection of human menstrual blood (MB), followed by the isolation and culture of mesenchymal stem cells (MSCs). Ethical clearance for the study was granted by the Institutional Ethics Committee (Pre-Clinical Studies), Department of Stem Cell Biology, Acadicell Innovations International Pvt. Ltd., Tamil Nadu, India**.** Menstrual blood samples were collected from healthy individuals ages 20-30

during the early days of the menstrual cycle with sterile silicone menstrual cups. 5ml of blood was added to a 50ml Falcon tube containing Hanks balanced salt solution (HBSS), 120 µg/mL Gentamicin sulfate, 2.7 µg/mL Amphotericin B, 80 µg/mL Vancomycin and 10 U/mL Heparin to make up 50ml volume. The Menstrual blood Mononuclear cells were separated by Ficoll-Paque gradient centrifugation at 600xg and washed in HBSS for two cycles. The Cell pellet was suspended and cultured in a T25 flask with 5ml complete media in sterile conditions. The cells were incubated in 5% CO2, at 37°C. After 24 hours, the growth medium was replaced with fresh complete medium to eliminate red blood cells and non-adherent cells, with subsequent media changes performed every three days. The cells were passaged after reaching 70-80% confluency[23–25]. Karyotyping, gene expression and flow cytometry characterizations were done to verify species authenticity, genomic stability and immunophenotype of the isolated MB-MSC.

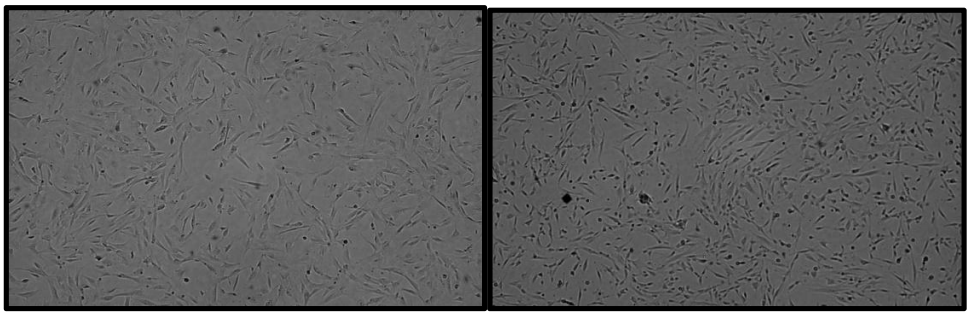

Figure 3. MB-MSCs at P1 and P2.

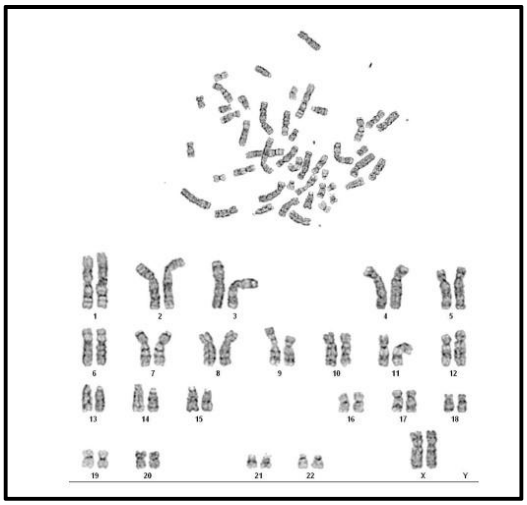

Figure 4. Karyotypic analysis of Menstrual blood mesenchymal stem cells (MSCs).

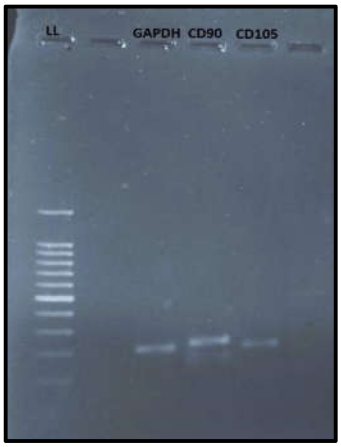

Figure 5. PCR analysis of MSC-specific gene markers.

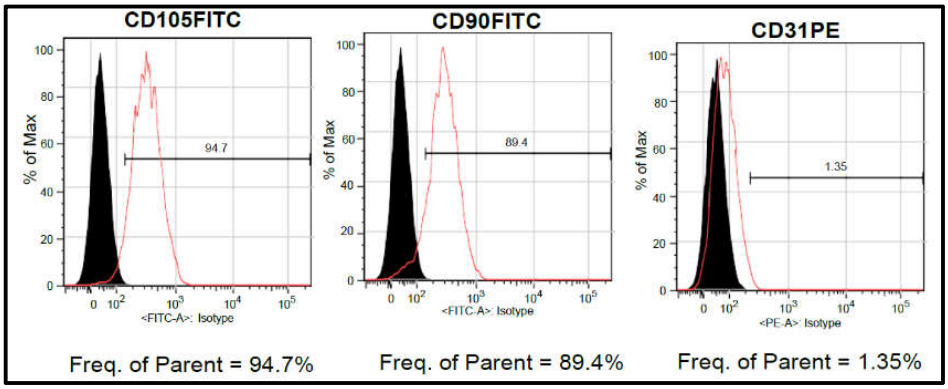

Figure 6. Flow cytometric characterization of MSC surface markers

2.3.2. Collection of Small Extracellular Vesicles (sEVs) and Priming of Sulphoraphane with sEVs

For sEVs isolation, at P2 the cells were subcultured in a T75 flask at cell seeding density of 4000 cells/cm2. Refer to figure 3. Once the cells reached 70-80% confluency, the media was removed and the cells were washed with PBS after which Dulbecco's Modified Eagle Medium (DMEM) supplemented with exosome-free FBS and Gentamicin sulphate was added and incubated for 48hrs to obtain sEVs and the conditioned media was collected. To remove cells and cell debris, the conditioned media was subjected to centrifugation at 300xg for 10 mins and 2000xg for 10 mins. Later the supernatant was centrifuged at 10,000xg for 30 min and the cell free supernatant was ultracentrifuged at 100,000xg for 120 mins at 4°C to collect the sEVs. The sEVs were suspended in PBS and used for further analysis[26,27].

SFN was dissolved in an organic solvent (DMSO). For loading SFN into sEVs, microwave-assisted method was used, where the exosome–drug mixture was briefly exposed to microwave irradiation. The microwave method works by using electromagnetic energy to temporarily loosen the lipid bilayer of the EVs by generating an oscillating electromagnetic field. This allows any small molecules SFN to diffuse into the Extracellular Vesicles. Once the electromagnetic field is stopped, the membrane reseals and SFN molecules are trapped inside. This mixture was used for further assays.

2.3.3. Characterization of SEVs

a) Nano Particle Tracking Analysis (NTA):

Nanoparticle tracking analysis (NTA) was used to determine the size distribution and concentration of the particles. Data acquisition and analysis were carried out using ZetaView. SFN-primed MB-MSC-derived sEVs were suspended in 1 mL of PBS and introduced into the sample chamber under controlled temperature conditions. Measurements were taken across 11 positions with two cycles per position using a 520 nm laser.

2.3.4. Invitro analysis using lung cancer cell line A549

a) Cytotoxicity assay

The MTT method was used to evaluate the cytotoxicity assay in order to assess the efficacy of SFN on A549 cells and its IC‰ value. A549 cells were planted at a density of 8 x 103 cells per well in a 96-well plate. To enable the cells to adhere, they were incubated for a full day. Fresh media with different SFN concentrations (0.5, 1.5, 2, 2.5, and 3μL) was added to the medium, and it was cultured for an additional 48 hours. The cells were then incubated for four hours after the addition of the MTT reagent, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. The number of living cells in the wells was then ascertained by measuring the absorbance at 570 nm using a microplate reader after DMSO was applied to dissolve the crystals and left in the dark for 30 minutes. The relative half maximum inhibitory concentration (IC50) was used to express cell viability. The results were presented as means and standard deviations for each experiment, which was carried out in triplicate.

b) Scratch assay

Scratch assay was done to determine the migratory properties of cells after being treated with SFN loaded sEVs. A549 cells were seeded into a 12-well plate to obtain a confluent monolayer. Following 24hrs incubation a scratch was made in a straight line in each well using a 1mL pipette tip. The cells were treated with SFN alone, sEVs alone, SFN loaded sEVs and with Serum free media as positive control and media with 10% FBS as negative control.

Observations of each plate was taken at the 0^{th} and 48^{th} hour where each experiment was conducted in duplicates and the results were analyzed using the ImageJ software.

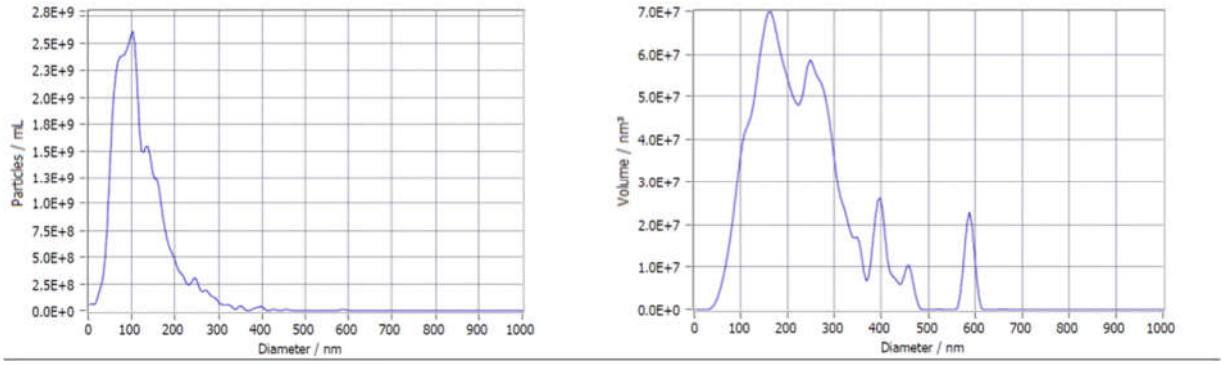

Figure 6: NT analysis of MSC derived EVs.

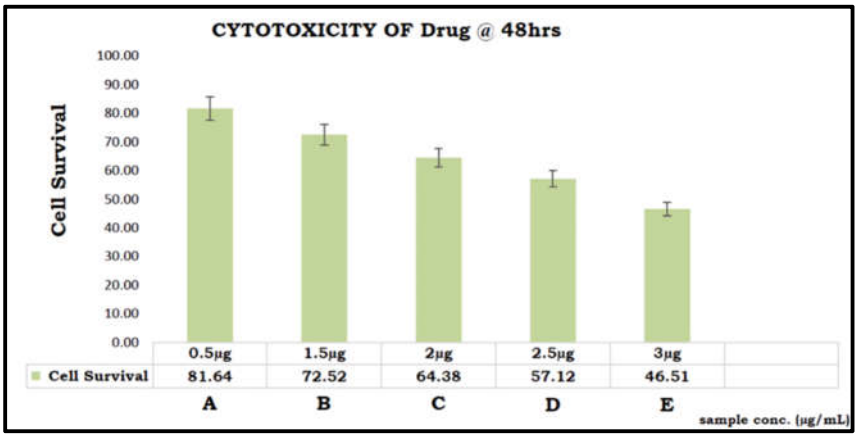

Figure 7: Cytotoxicity assay of A549 cell line with drugs and sEVs.

Table 1: Results of the scratch assay – rate of proliferation (%).

Sample	0th hour area(μm²)	48th hour area(μm²)	Proliferation rate%	Inhibition%
Positive Control	566,930	333,258	41%	58%
Negative Control	567,087	508,745.3	10%	89%
MSC- sEVs	595,721.3	548,977.3	7%	92%
SFN	634,604	611,670.7	3%	96%
SFN primed sEVs	682,687.7	849,816.7	-24%	124%

3. RESULTS

3.1.Characterization of MB-MSCs using cytogenic analysis:

Cytogenetic analysis of MB-MSCs revealed a normal female diploid karyotype (46,XX) and no evidence of structural or numerical chromosomal aberrations as shown in Figure 4. The karyotype showed no abnormalities, confirming that the MB-MSCs retained genomic stability in culture.

3.2. Characterization of MB-MSCs using PCR-based Authentication:

PCR was done to confirm the expression of MSC markers in the MB-MSCs. As shown in Figure 5, from left to right, the gel lanes correspond to: DNA ladder, GAPDH (positive control), CD90, and CD105. Distinct amplification bands were observed for GAPDH, CD90, and CD105 at their expected sizes, confirming positive expression of mesenchymal markers.

3.3. Characterization of MB-MSC using flow cytometry:

Flow cytometry was used to examine the surface marker profile of MB-MSCs. As shown in Figure 6, the cells were strongly positive for CD90 and CD105 which are MSC markers. In contrast, CD31 which is a marker for hematopoietic cells, remained at baseline, indicating little to no expression. This is consistent with the expected immunophenotype of MSCs and supports the identity of the isolated population.

3.4.Characterization of sEVs Using Nanoparticle Tracking Analysis (NTA)

To determine the size distribution and concentration of the MB-MSC derived sEVs Nanoparticle tracking analysis (NTA) was performed using ZetaView. As shown in Figure 6, this analysis revealed that the isolated vesicles were 50–195 nm in size, with the majority of the vesicle population in the diameter size range of 90–110 nm and the mean particle size of MSC-derived sEVs was 119.6 nm. The concentration of MSC-derived sEVs was quantified as 5.8×10^{13} particles/mL.

3.5.Invitro Analysis using Lung Cancer cell line – A549

3.5.1. Cytotoxicity assay

The cytotoxic activity of the sample was assessed against A549 lung carcinoma cells using the MTT assay. As shown in Figure 7, treatment with increasing concentrations (0.5–3.0 µg/mL) for 48 hours resulted in a dose-dependent reduction in cell viability. Cell survival decreased from 81.6% at 0.5 µg/mL to 46.5% at 3.0 µg/mL. The calculated IC_{50} value was approximately 2.3 µg/mL. In our study, we found that the proliferation of A549 cells was drastically reduced by SFN.

3.5.2. Scratch assay

The concentration of MSC-derived sEVs and SFN-primed MSC-sEVs used in the study was standardized to 5 µL, based on their proliferative effect in preliminary assays. This same concentration was applied in the scratch assay to evaluate their impact on lung cancer cell migration. The experimental groups and their corresponding proliferation rates (%) and inhibition rates (%) are summarized in Table 1. Measurements of scratch closure were recorded at 0 and 48 hours. The proliferation rate (%) is calculated using:

$$\text{Proliferation rate (\%)} = \frac{\text{Initial measurement at 0hrs- measurement at 48hrs}}{\text{Measurement at 0hrs}} \times 100$$

The results demonstrated a marked reduction in proliferation for cells treated with SFN-primed MSC-sEVs, which showed the lowest migration rate among all groups. Notably, this inhibitory effect was more pronounced than that observed with unprimed MSC-sEVs and SFN alone highlighting the enhanced anti-proliferative efficacy of drug priming in combination with EV delivery.

4. Discussion

Extracellular vesicles, particularly small Extracellular Vesicles, were initially regarded as simple mediators of intercellular communication, with their role thought to be limited to the transfer of signaling molecules between cells. However, subsequent research has revealed their involvement in a much wider range of physiological and pathological processes. The last few years has seen a recent boom in the research sEVs due to their biocompatibility, drug delivery, decreased toxicity, circulatory stability and tumor specificity[28].

EVs are natural nanocarriers that reflect the molecular profile of their donor cells, carrying proteins, lipids, and nucleic acids that influence their therapeutic role in gene and immune therapies. For example, immune cell–derived Extracellular Vesicles can enhance antitumor activity by presenting MHC molecules and co-stimulatory signals that activate T cells[29]. Tumor cells often evade immune clearance by overexpressing CD47, which binds to signal

regulatory protein α (SIRPα) on macrophages and delivers a 'don't eat me' signal that suppresses phagocytosis. Extracellular Vesicles engineered to carry modified SIRPα can disrupt this interaction, restoring macrophage-mediated engulfment of tumor cells. In addition, SIRPα-enriched small Extracellular Vesicles have been shown to enhance T-cell infiltration in mouse models, contributing to suppressed tumor growth[30].

The molecular makeup of Extracellular Vesicles (EVs) is largely shaped by their cell of origin and the pathway through which they are generated, which in turn dictates their effects on recipient cells. Lipid composition profiling of EVs provides valuable clues about their cellular source and biogenetic pathways[17]. Almost every cell releases EVs, so other than just therapeutic purposes, the circulating cancer cell–derived EVs offer a noninvasive means for early cancer diagnosis and screening, enabling timely therapeutic intervention.[31]

MSCs are increasingly recognized for their therapeutic potential, largely due to their strong migratory and homing capacity. Studies, including xenograft mouse models of brain tumors, have demonstrated this tumor-tropic behavior, which is thought to be mediated by chemokines such as MCP-1, SDF-1, and SCF-1, although the exact mechanisms remain unclear[32]. In a pioneering study using in vivo bioluminescent imaging, systemically administered MSCs were shown to migrate selectively to sites of inflammation, such as wounds and tumors, in both syngeneic and xenogeneic mouse models[33]. This selective engraftment was driven by inflammatory cues, with MSCs incorporating into the local microenvironment and in some cases persisting long after tissue repair. Importantly, tumor models confirmed MSC accumulation within the tumor stroma, supporting their potential as targeted delivery vehicles and diagnostic tools. Engineered neural stem cells (NSCs) were shown to express and secrete intact anti-HER2 antibodies, enabling targeted delivery to CNS metastases and invasive tumor regions that trastuzumab cannot reach. Antibody localization was restricted to tumor sites while maintaining therapeutic activity, underscoring the potential of Stem cell-based platforms to enhance antibody therapy for solid tumors[34].

RNAs are key components of EVs, with non-coding RNAs (ncRNAs) such as miRNAs, circRNAs and lncRNAs being the most enriched species. In MSC-derived EVs, ncRNAs play crucial regulatory roles in cancer development and progression. Notably, EV-associated miRNAs have shown therapeutic potential across multiple cancers, including breast, liver, ovarian, and pancreatic malignancies[17]. EVs contain diverse bioactive molecules all of which play key roles in mediating intercellular communication. Mesenchymal stem cell-derived EVs have been shown to suppress tumor development through paracrine signaling, an effect largely attributed to tumor-associated miRNAs carried within these vesicles. Human bone marrow MSC (BMSC)-derived EVs have been shown to inhibit angiogenesis and breast cancer progression by transferring miRNAs such

as miR-16 and miR-100 into tumor cells causing to VEGF suppression. This regulation occurs partially through direct VEGF targeting as well as modulation of the mTOR/HIF-1α signaling pathway, highlighting the role of EV miRNAs in reprogramming the tumor microenvironment[35]. Gene-modified MSC-EVs enriched with miR-497 were shown, using microfluidic tumor models, to suppress Non-Small Cell Lung Cancer (NSCLC) growth, migration, and angiogenesis by acting on both endothelial and cancer cells[36].

Several studies have been reported using EVs as delivery vehicles for drugs in treatment of various cancers. Paclitaxel was loaded onto MSC-sEVs and showed anti-proliferative activity on CFPAC-1 and also to overcome multi drug resistance cancers[37,38]. Doxorubicin was loaded onto sEVs and used to deliver the drug to tumor tissue in BALB/c nude mice[39]. Clinical trials have explored the therapeutic potential of EVs as cancer vaccines. In one study with 41 participants, dendritic cell–derived sEVs were used to deliver tumor-associated proteins, including MART-1 and MAGEA1 in patients with non–small cell lung cancer (NSCLC). When combined with induction chemotherapy, this approach stimulated antitumor immune responses and slowed disease progression. Notably, the phase II trial (ClinicalTrials.gov Identifier: NCT01159288) reported an improvement in patient survival[40].

Given the growing evidence supporting the therapeutic potential of sEVs, our study focuses on isolating sEVs derived from human menstrual blood–mesenchymal stem cells (MB-MSCs) for application in lung cancer therapy. These vesicles possess tumor-homing capabilities and strong immunomodulatory properties, which make them highly attractive as natural nanocarriers. By leveraging these unique characteristics, MB-MSC-sEVs may offer an innovative and effective strategy for the development of alternative treatment modalities against lung cancer.

The MSCs derived from menstrual blood was characterized by Karyotyping which confirmed the genomic stability of the MB-MSCs, showing a normal chromosomal profile without detectable abnormalities. Flow cytometry analysis confirmed their mesenchymal identity, with strong expression of CD90, and CD105, and the absence of hematopoietic marker CD31. Gene expression profiling further validated these findings, demonstrating the upregulation of mesenchymal stem cell–specific genes. The sEVs collected from the MSCs were characterized by NTA, wherein the size range was mostly 90–110 nm.

The anti-cancer effects of MSC-derived sEVs and SFN-primed sEVs were confirmed through in vitro studies. Our findings indicate that a final concentration of 5 µL of MSC-sEVs was sufficient to reduce A549 cell viability, as reflected in the proliferation and cytotoxicity assays. SFN alone has shown cytotoxicity in A549 cells. In line with this, our

study demonstrates that the combination of MSC-EVs with SFN yielded strong anti-cancer effect in vitro.

5. CONCLUSION

Drug-loaded small Extracellular Vesicles are a potential therapeutic option for cancer treatment. The overall results of our study showed anti-cancer activity against A549 lung cancer cell line. These findings provide proof-of-concept that sEVs can serve as highly effective carriers for drug delivery, owing to their natural stability, biocompatibility, and ability to transport therapeutic molecules directly to target cells while minimizing off-target effects while also overcoming the drawbacks of conventional cancer therapies. Our findings also suggest that the phytochemical drug Sulphoraphane exhibits strong biocompatibility, supporting its potential as an innovative therapeutic approach against lung cancer proving Sulphoraphane loaded MSC-sEVs are a suitable option for further clinical applications. While the compound demonstrates promising anti-cancer effects, the exact molecular mechanisms underlying its activity remain to be elucidated. Moreover, as the field of Extracellular vesicle biology is still in its infancy, extensive studies are required to validate and translate this approach into clinical practice.

AUTHOR'S CONTRIBUTION

All authors contributed equally.

ETHICAL APPROVAL AND CONCENT TO PARTICIPATE:

The study protocol was approved by the Institutional Ethics Committee (Pre-Clinical Studies), Department of Stem Cell Biology, Acadicell Innovations International Pvt. Ltd, Tamil Nadu, India with approval number IEC/C-P/3/2025 and performed in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained because of the retrospective nature of this study.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY

Data shall be provided upon request.

USE OF ARTIFICIAL INTELLIGENCE (AI) – ASSISTED TECHNOLOGY

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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