Flavonolignans-routed synthesis of selenium nanowires (SeNWs) and their cytotoxicity, cell migration potential against triple negative breast cancer cells

1 | INTRODUCTION

Breast cancer is the second most leading cause of deaths in women after lung cancer. Breast cancer is the most common type of cancer in women population among them. Triple negative breast cancer (TNBC) refers to the cancer type where the estrogen, herceptin and progesterone receptors are absent in their phenotype. Approximately, 1.2 million cases of breast cancer are diagnosed worldwide every year. Of these, 12–20% are exclusively triple-negative phenotype. Triple negative breast cancer type has minimal treatment option than any other metastatic cancer. Due to lack of oestrogen or progesterone or Herceptin receptors for targeted drug delivery [1].

Nanomaterials are nowadays progressively incorporated in our daily life and environment as in the form of pharmacology, industrial, agricultural, medical, cosmetic and food products. Moreover, it has been reported that nanomaterials can negatively affect cancer cell viability and physiological process [2]. Furthermore, more insight is sought on the expected effects of nanomaterials on cells at the molecular level, such as the production of reactive oxygen species after the entry into the cell [3] and consequential cause DNA damage [4] leading to apoptosis and cell cycle arrest [5]. A better understanding of nanobiomolecular interactions may provide more information for future efforts to develop safe mode for anticancer drug designing. Recent studies have shown that selenium-based nanomaterials (SeNPs) can significantly reduce the migration/invasion level of cancer cells and suppress further metastasis. Selenium is an essential component of human diet and a number of studies have confirmed their pharmacological and therapeutic properties against various cancers. However, at elevated doses, selenium compounds usually turn the oxidative cellular process that triggers cell growth inhibition properties. Thus, the uses of Se compounds for anticancer therapy have been to a great extent during the last decade and results of studies have shown that Se molecules have reduced the risk of various types of cancer progression [6].

For cell migration process, signal transduction from the extracellular compartment to the cytoplasm requires many vital molecules as cell surface transducers. These molecules react with the extracellular stimuli, and thus activate the cell migration process. However, whether selenium nanowires affect the migration and invasion potential of cancer cells remains to be studied. The focus of our current study is to determine the cellular mechanism by which SeNWs affects the migration and invasion potential of triple negative breast cancer cells (MDA-MB-231). Our finding confirms that SeNWs down regulate the expression of cellular skeleton and inhibits the migration and invasion potential of cancer cells, provides new insights into the possible mechanisms of the anti-metastatic potential of selenium nanowires.

2 | EXPERIMENTAL SECTION

Silymarin (C25H22O10; CAS No. 65666-07-1) was purchased from Sigma Chemicals, India. For synthesis of Se nanowire, 10 M concentration of 50 mL silymarin solution was prepared in the presence of sodium selenate solution, stirred for 8 h at room temperature and incubated (30 °C, 24 h). The final Se nanowires were purified through consequent centrifugation, washing with 0.9% NaCl, sonication, washing with Tris-HCl containing sodium dodecyl sulphate (SDS) and finally isolation with water-octanol systems. Silymarin-routed synthesis of selenium nanowires were characterized with X-ray crystallographic method. The X-ray crystallography of the silymarin-mediated synthesis of nanowires was studied using a Philips PW 17291 powder X-ray diffractometer with a voltage of 40 kV and current 25 mA. The scanning rate employed was 1 deg/min. The synthesized SeNWs were placed on polycarbonate substrate and the excess water was left to dry at room temperature. They were then dried in a critical point dryer using carbon dioxide, and sputter coated with gold in a metallizer, and examined under a scanning electron microscope (JSM5600LV, JEOL, Japan).

MDA-MB-231 cells were cultured in DMEM culture medium (l-glutamine and Phenol Red) (HiMedia) supplemented with 10% (v/v) heat-inactivated foetal bovine serum (FBS) (HiMedia), and 100 μg/mL of penicillin and 10 μg/mL of pencillin and 10 μg/mL
of streptomycin (HiMedia). Cells were cultured at 37 °C and 5% CO₂ atmosphere. The cell viability of SeNWs was assessed by MTT assay. In brief, the MDA-MB-231 cell lines were grown in a 96-well plate and treated with various concentrations of SeNWs at 37 °C for 24 h. The cells were further incubated with MTT solution (2 mg/mL) for another 4 h. The supernatants were then flicked off and dissolved in 100 μL of DMSO. The absorbance was determined at 490 nm using a microplate reader (Multiscan Reader-United States of America) [7]. A suspension of MDA-MB-231 was seeded into culture- inserts within the medium, in order to allow the creation of a homogeneous width, cell-free space in the middle of a confluent cell monolayer. Twenty-four hours after seeding, the inserts were removed, and photographs of the resulting scratches were taken using phase-contrast microscopy at T0. The cells were cultured for a further 24 h. Cell migration was evaluated by measuring the cell-free surface at the beginning of the experiment (T0) and the surface covered by the cells at the end of the experiment with Image J Software. The results were expressed as the percentage of wound closure in each condition. To evaluate cell apoptosis, Annexin-V apoptosis detection kit (BD Biosciences, United States of America) was employed. Simply, different concentration SeNWs-pre-treated cells were trypsinized and suspended in 300 μL of 1X binding buffer. Then, 5 μL of Annexin-V was added for 15 min and mixed with 5 μL propidium iodide (PI) solution for another 5 min. At last, 200 μL of 1X binding buffer was added and analysed by a flow cytometer (BD Biosciences, United States of America) [7].

3 | RESULT AND DISCUSSION

Flavonolignans-routed synthesis of SeNWs was characterized with XRD Raman spectrum and SEM analysis. The XRD pattern, SEM images of SeNWs and Raman spectra of SeNWs are shown in Figure 1(a–d). All the diffraction peaks could be recorded as trigonal phase of selenium with lattice constants of \( a = 4.367 \) Å and \( c = 4.956 \) Å, which were in acceptable
concurrence with the revealed information (JCPDS card No. 06–0362, $a = 4.3662$ Å and $c = 4.9536$ Å). Simultaneously no different lattice constants of impurities were distinguished. Contrasted with the standard example of t-selenium, the intensities of the (100) and (110) diffraction peaks were extraordinarily improved, which showed that these Se nanowires had been specially developed along with the (001) direction [8]. No XRD peaks arising from impurities could be detected, indicating that only elemental selenium grains with high crystallinity and purity were obtained. The Raman spectrum provides further evidence confirming the trigonal phase of SeNWs. Figure 1(b) shows a typical Raman spectrum of silymarin-routed synthesis of Se nanowires. Only one resonance peak at around $240 \text{ cm}^{-1}$ was observed, which is attributed to the vibration of helical selenium that exists in the trigonal phase, indicated that the as-prepared SeNWs has high quality crystallinity. The morphologies of the prepared SeNWs were examined by scanning electron microscopy techniques. Figure 1(c) shows a representative SEM image of the prepared nanowires. It revealed the general morphology of the Se nanowires. Previous study stated that over 99% proportion of synthesized selenium nanomaterials showed wire structure with a mean diameter of 65 nm and lengths of over more than a few micrometres [9]. In addition to that, an increase in the Se concentration may cause a decrease in the uniformity of the Se nanowires, whereas higher silymarin content would favour the formation of uniform SeNWs.

To evaluate the cytotoxic potential of SeNWs, we examined the cell proliferation by MTT assay. As illustrated in Figure 2(a), cells were treated with SeNWs at different doses for 48 h, and we found that the inhibitory concentration (IC$_{50}$) synthesized Se nanowires determined at $25 \mu g/mL$, further the anticancer activity showed dose-dependent manner. The results showed that there was significant inhibition between the control group and treated groups at 24 h. In addition, morphological analysis revealed the size of the cells were shrinking and floated in treated group Figure 2(b). Recent report confirms that cytotoxicity analysis of the nano-selenium showed direct dose-response relationship, in which human melanoma (A375) cell viability decreased at the higher concentrations [10].

Cancer cell migration plays a vital role in disease progression, thus the effects of SeNWs on migration ability of MDA-MB-231 cells. As shown in Figure 3, SeNWs inhibited the cell migration significantly. When compared to the control group, SeNWs noticeably decreased the migratory potential from 92% to 45%. Mechanical rigidity of cancer cells has been shown to metastatic potential in patient tumour cells as well as in cultured cancer cell lines. Lower stiffness is related to more invasive cells. Cell migration is a difficult biological process, involving turnover of cell-matrix adhesion molecule signalling pathway, and also the internal cytoskeleton that pulls the cell and directs cell migration [11]. Collectively, these results suggested that SeNWs inhibited migration of MDA-MB-231 cells by inhibiting the structural cellular skeleton.

To investigate potential of SeNWs on apoptosis in MDA-MB-231 cells, flow cytometry was employed. After cells were treated with various concentrations of SeNWs for 24 h, after cells were stained with Annexin-V/PI. The flow cytometric histograms provided the corresponding distribution of the cells, when the MDA-MB-231 cells were treated with the SeNWs, and apoptosis was increased from upto 42%. Treated group cells were shown 22% and 14.2% of pro and late apoptotic cells whereas only 2.06% and 1.25% apoptotic cells were observed in the control groups (Figure 4).

Enhanced apoptotic activity of selenium nanoparticles has been studied [12], Furthermore MEP-Se NWs also be considered for entomological and biomedical applications, with extraordinary reference to the administration of biofilm shaping microbial microorganisms and arbovirus mosquito vectors [13] and the obtained data shows SeNPs significantly inhibited MDA-MB-231 cell proliferation with the low cytotoxicity against normal cells, and noticeably inhibit the aggression and migration of MDA-MB-231 cells.

4 | CONCLUSION

Biologically potential (flavonolignans) silymarin-mediated SeNWs showed promising cytotoxic and inhibited cell migratory activity against MDA-MB-231 cells. SeNWs are found to be potent cytotoxic agent and anti-metastatic under in vitro conditions. The exact inhibitory potential and molecular
FIGURE 3  SeNWs-induced inhibition of cell migration in MDA-MB-231 cells. Cells were seeded overnight prior to the creation of scratches with p200 pipet tips. The cells were then exposed to various concentrations (a) untreated control; (b) 25 μg/mL; (c) 50 μg/mL; (d) 100 μg/mL concentration. Data are presented as the means ± SD of three independent experiments in each group.

FIGURE 4  Apoptotic analysis by flow cytometry using PI and Annexin-V double staining method. (a) untreated control; (b) 25 μg/mL; (c) 50 μg/mL; (d) 100 μg/mL concentration treated MDA-MB-231 cells. The cell populations shown at lower left, lower right, upper right, and upper left represent living cells, early apoptotic cells, late apoptotic and necrotic cells, respectively. Data are presented as the means ± SD of three independent experiments in each group.
mechanism of synthesized SeNWs remain unclear. This paper revealed that SeNWs significantly suppressed cell viability and migration likely to human triple negative breast cancer cells. Moreover, SeNWs significantly induce the apoptotic induction in triple negative breast cancer cells. Nevertheless, in-depth studies should be conducted to investigate the anticancer mechanism of SeNWs.

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