Isolation and characterization of exosomes derived from saffron petals and investigation of their role in cell migration

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Introduction

Plant-derived exosomes and extracellular vesicles have recently gained significant attention as natural, biocompatible nanoscale carriers with promising applications in cancer research. These vesicles are capable of encapsulating and transporting a variety of bioactive plant-derived molecules, including polyphenols, flavonoids, and antioxidant compounds, to target cells. Saffron petals, often considered an agricultural by-product, are known to contain high levels of antioxidant and biologically active substances, making them a valuable source for the isolation of extracellular vesicles with potential therapeutic relevance. In breast cancer, particularly the highly invasive MDA-MB-231 cell line, processes such as uncontrolled proliferation, enhanced migratory capacity, oxidative stress imbalance, and treatment resistance play central roles in disease progression. Therefore, evaluating the biological influence of natural nanoparticles on the behavior of such cancer cells is of considerable importance. The present study focuses on the extraction, characterization, and biological assessment of extracellular vesicles derived from saffron petals, aiming to provide deeper insight into their potential therapeutic applications against cancer.

Methods

Extracellular vesicles (EVs) were isolated from saffron petals using a multistep differential centrifugation method and resuspended in PBS. EV morphology was examined by FE-SEM, their size distribution was measured using DLS, and zeta potential and protein concentration were determined through standard analyses, including the BCA assay. Antioxidant activity was assessed using the FRAP assay at concentrations of 0.1, 0.5, and 1 mg/ml.. The human breast cancer cell line MDA-MB-231 was cultured under standard conditions (37°C, 5 percent CO_2).

EV effects on cell viability were evaluated using the MTT assay at the same concentration range. To assess migration, a scratch assay was performed on confluent monolayers, followed by EV treatment and imaging at designated time points.

Results

Characterization analyses confirmed that the saffron petal–derived extracellular vesicles (EVs) possessed spherical to oval morphology and nanoscale dimensions, with FE-SEM showing an average size of 58.37 ± 12.92 nm and DLS indicating a distribution within 80–140 nm. The vesicles carried a negative surface charge (zeta potential: −12.2 mV) and exhibited measurable protein content (11.5 µg/ml). The biological effects of EVs on the human breast cancer cell line MDA-MB-231 demonstrated a clear dose-dependent cytotoxic pattern. MTT analysis showed that treatment with 0.5 mg/ml EVs reduced cell viability substantially, with approximately half of the cancer cells becoming non-viable, while 1 mg/ml further intensified the inhibitory effect. In contrast to normal fibroblasts, EV exposure did not enhance viability in cancer cells at any concentration tested. Migration analysis using the scratch assay further supported the inhibitory influence of EVs on cancer cell behavior. Although MDA-MB-231 cells typically display rapid migratory capacity, EV treatment reduced wound-closure ability compared to untreated controls, indicating impaired migration following exposure.Overall, the results confirm that saffron petal–derived EVs exhibit selective cytotoxicity and migration-inhibitory effects toward MDA-MB-231 cancer cells.

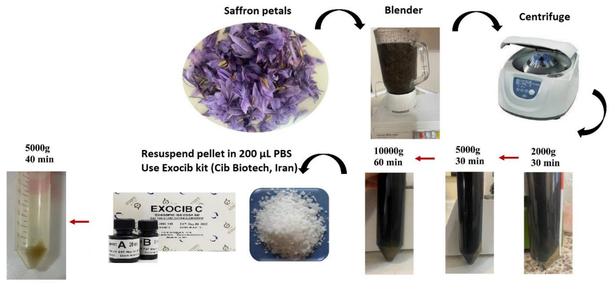

Figure 1. Schematic representation of the EVs isolation process from saffron petals. Fresh petals are washed, homogenized, and subjected to sequential centrifugation and filtration steps to remove debris and large particles. EVs are finally pelleted via resuspended in sterile PBS for downstream analyses.

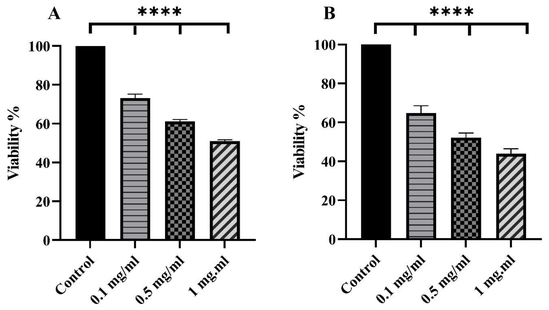

Figure 3. Evaluation of cell viability of MDA-MB-231 cells after treatment with saffron petal-derived EVs at different concentrations (0.1, 0.5, and 1 mg/ml) using the MTT assay at 48 hours (A) and 72 hours (B). Cell viability decreased in a dose-dependent manner, and at 0.5 mg/ml, approximately half of the cells were non-viable compared to the control. ****p < 0.0001

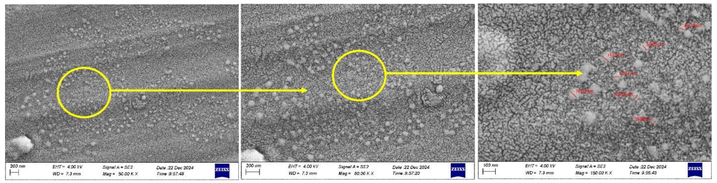

Figure 2. FE-SEM images of saffron petal-derived EVs showing their spherical to oval morphology and uniform size distribution. The vesicles exhibit an average diameter of approximately 58 nm, and the integrity of the bilayer membrane is maintained after glutaraldehyde fixation.

Conclusions

This study shows that extracellular vesicles derived from saffron petals possess meaningful anticancer activity against MDA-MB-231 breast cancer cells. The vesicles exhibited stable nanoscale characteristics and measurable antioxidant properties, indicating their biological functionality. In cell-based assays, they reduced cancer-cell viability in a clear dose-dependent manner, with the strongest inhibitory effect observed at 0.5 and 1 mg/ml. Additionally, EV treatment impaired the migratory capacity of this aggressive cell line, suggesting a potential role in limiting metastatic behavior. Overall, saffron petal–derived EVs appear to be natural, biocompatible nanostructures with promising applications for complementary anticancer strategies, warranting further mechanistic and in-vivo investigation.