Cytotoxic, Apoptotic, and Antiproliferative Effects of Raphanus sativus Extract on MDA- MB-231 Cells: A Translational Perspective for Exercise Therapy in Cancer Rehabilitation

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Article

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Abstract

Objective:

This study aimed to evaluate the anticancer potential of Raphanus sativus methanolic extract (RME) in triple-negative breast cancer (MDA-MB-231) cells and to provide a novel translational perspective by linking the findings with physiotherapy-based oncological rehabilitation.

Methods:

Cytotoxic, apoptotic, and antiproliferative effects of RME were simultaneously assessed using MTT assay, Annexin V staining, and AgNOR analysis. Cell viability, apoptosis induction, and nucleolar activity were quantified following RME exposure across a range of concentrations.

Results:

RME significantly reduced cell viability in a dose-dependent manner (IC_{50} = 140.5 µg/mL), induced apoptosis, and suppressed proliferative activity. To our knowledge, this is the first study to combine these three complementary methods in the evaluation of Raphanus sativus extract. Beyond cellular findings, apoptosis induction and reduced proliferative activity conceptually align with physiotherapy goals by mitigating systemic inflammation, cancer-related fatigue, and cachexia, while supporting muscle strength preservation and functional independence. Moreover, radish-derived phytochemicals are known to reduce oxidative stress and mitochondrial dysfunction, paralleling the physiological effects of aerobic and resistance exercise interventions.

Conclusion:

This study demonstrates that RME exerts significant cytotoxic, apoptotic, and antiproliferative effects in MDA-MB-231 cells. Its originality lies not only in the methodological novelty but also in being the first to propose a translational framework linking phytotherapy with physiotherapy. Although limited to in vitro data, these findings provide preliminary evidence and an innovative interdisciplinary perspective, encouraging future studies to investigate integrated phytotherapy–physiotherapy strategies in cancer rehabilitation.

INTRODUCTION

Breast cancer is the most common malignancy among women worldwide and remains one of the leading causes of cancer-related mortality. Despite substantial advances in therapeutic strategies, prognosis remains poor in aggressive subtypes such as triple-negative breast cancer (MDA-MB-231), where survival rates are significantly reduced and resistance to conventional therapies is frequent [1]. Accordingly, the development of novel therapeutic targets and supportive strategies is of paramount importance, not only to improve disease control but also to enhance overall quality of life.

Cancer treatment should not be limited to tumor eradication but must also address the prevention of therapy-induced functional decline and the promotion of quality of life. In this context, physiotherapy and rehabilitation have become integral to multidisciplinary cancer care [2]. Clinical evidence demonstrates that physiotherapy contributes to reducing cancer-related fatigue, preserving exercise capacity and muscle strength, supporting pulmonary function, managing lymphedema, alleviating pain, and improving psychosocial well-being [3,4]. Thus, oncological rehabilitation is now recognized as a complementary and indispensable component of comprehensive cancer management [5,6].

Parallel to these clinical efforts, interest in the integration of phytotherapeutic agents into cancer care has grown rapidly. Natural compounds are reported to exert anti-inflammatory and antioxidant properties, while also modulating key cellular processes such as apoptosis and proliferation, often with lower toxicity compared to chemotherapeutic agents [7–9]. Among cruciferous vegetables, *Raphanus sativus* (radish) is particularly rich in glucosinolates and isothiocyanates, which have demonstrated hepatoprotective, antioxidant, and antitumoral effects in preclinical studies. Importantly, excessive generation of reactive oxygen species (ROS) plays a pivotal role in carcinogenesis by inducing DNA damage, chronic inflammation, and mitochondrial dysfunction. Phytochemicals contained in radish extracts have been shown to suppress ROS production, exert cytoprotective activity, and trigger apoptosis via caspase activation and modulation of β-catenin signaling [10,11]. These mechanisms collectively suggest that radish may act as a promising adjunct in anticancer strategies.

From a methodological perspective, nucleolar organizer regions (NORs), which consist of transcriptionally active rDNA and associated proteins, are considered sensitive markers of cellular proliferation. Their visualization through silver staining (AgNOR) reflects ribosomal biogenesis and proliferative activity, thereby offering a useful surrogate marker for evaluating the antiproliferative potential of novel therapeutic agents [12–15].

To the best of our knowledge, no previous study has investigated the apoptotic and antiproliferative effects of radish methanolic extract (RME) in triple-negative breast cancer cells using AgNOR staining methods. Integrating phytotherapy into physiotherapy-based approaches requires a clear understanding of the cellular mechanisms of active compounds before translation to human studies. Unlike previous in vitro research that primarily reported cytotoxic outcomes, the present study was designed to interpret the cellular effects of RME from a physiotherapy-oriented perspective. Specifically, we aimed to link cellular responses such as oxidative stress reduction, apoptosis, and antiproliferative activity with functional outcomes relevant to rehabilitation (e.g., preservation of exercise capacity, muscle strength, and overall functional status). By doing so, this research provides a translational framework that bridges molecular findings with physiotherapy practice and offers a scientific foundation for future studies exploring the integration of phytotherapy and physiotherapy in cancer rehabilitation.

MATERIALS AND METHODS

Cell Line and Reagents

The human triple-negative breast cancer cell line MDA-MB-231 was obtained from the Department of Anatomy, Erciyes University Faculty of Medicine (Kayseri, Turkey). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Sartorius, Göttingen, Germany) supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine, and 1% penicillin/streptomycin. Cells were maintained at 37°C in a humidified incubator with 5% CO_2. The Annexin V-FITC apoptosis detection kit was purchased from Luminex Corporation (Austin, TX, USA). All other chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise specified [16].

Preparation of Radish Methanolic Extract (RME)

Fresh radishes (*Raphanus sativus*) were obtained from a local market in Kayseri, Turkey. Plant material was washed, dried, and ground into powder. Extraction was performed using 70% methanol at room temperature for 24 h, and the process was repeated four times. The combined extracts were filtered, concentrated under reduced pressure at 37–38°C using a rotary evaporator (Buchi Rotavapor, Switzerland), and subsequently lyophilized. The lyophilized extract was stored at − 20°C until further use [17–19].

Cell Culture and Treatment

For all experiments, MDA-MB-231 cells were used at passage number 22. Cells were seeded into appropriate culture plates and allowed to adhere overnight before treatment. RME was dissolved in dimethyl sulfoxide (DMSO) and diluted in culture medium to final concentrations of 0, 50, 100, and 200 µg/mL. The final concentration of DMSO did not exceed 0.1% in any treatment condition [20].

Cytotoxicity Assay (MTT Test)

Cell viability was assessed using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. Briefly, MDA-MB-231 cells were seeded into 96-well plates (1×10^4 cells/well) and allowed to attach overnight. Cells were then exposed to increasing concentrations of RME (0–200 µg/mL) for 24 h. Following treatment, 10 µL of MTT solution (5 mg/mL) was added to each well, and the plates were incubated for 4 h at 37°C. The resulting formazan crystals were dissolved in 200 µL of DMSO, and absorbance was measured at 490 nm using a microplate reader (Promega, Madison, WI, USA). Cell viability was expressed as a percentage of untreated controls, and experiments were performed in triplicate [21].

Apoptosis Detection (Annexin V-FITC/PI Staining)

Apoptotic cell death was quantified using the Annexin V-FITC/propidium iodide (PI) double-staining method. MDA-MB-231 cells were seeded into 6-well plates at a density of 1×10^5 cells/well and incubated overnight. Cells were then treated with RME (0, 50, 100, 200 µg/mL) for 24 h. After treatment, cells were harvested, washed twice with cold PBS, and stained with Annexin V-FITC and PI according to the manufacturer's instructions. The percentage of apoptotic cells was determined using the Muse Cell Analyzer (Luminex, Austin, TX, USA) [22].

AgNOR Staining and Quantification

The antiproliferative effects of RME were assessed by evaluating nucleolar organizer regions (NORs) using silver staining. After treatment with RME (0, 50, 100, 200 µg/mL), MDA-MB-231 cells were harvested, spread on clean glass slides, air-dried, and fixed in methanol:acetic acid (3:1, v/v). AgNOR staining was performed using the method described by Ploton et al. (1986) [23]. Stained nuclei were examined under a light microscope (Eclipse 80i, Nikon, Tokyo, Japan), and images were captured with a digital camera (DS-Fi1, Nikon). Quantitative analysis was performed using ImageJ software (version 1.47t, NIH, Bethesda, USA). For each sample, 50 nuclei were randomly selected, and both the mean AgNOR number and the total AgNOR area per nuclear area (TAA/NA) were calculated [24].

Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (IBM Corp., Armonk, NY, USA) for Windows 22.0. The Shapiro–Wilk test was used to assess the normality of data distribution, and Levene's test was applied to evaluate the homogeneity of variances. Since the data met the assumptions of normality and homogeneity ($p > 0.05$), parametric tests were applied. Group comparisons were performed using one-way analysis of variance (ANOVA). Post-hoc pairwise comparisons were conducted using Tukey's test. Data are expressed as mean ± standard deviation (SD), and $p < 0.05$ was considered statistically significant.

RESULTS

Cell Viability

The cytotoxic effects of RME on MDA-MB-231 cells were evaluated using the MTT assay. Cells were treated with increasing concentrations of RME (0–500 µg/mL) for 24 h. A significant, dose-dependent decrease in cell viability was observed at all tested concentrations compared with the control group ($p < 0.001$) (Fig. 2). The IC_{50} value of RME in MDA-MB-231 cells was calculated as 140.5 µg/mL (Fig. 3).

Apoptosis

To further assess whether the cytotoxic effect of RME was associated with apoptosis, Annexin V-FITC/PI staining was performed. Representative flow cytometry plots of apoptosis induction at different concentrations are shown in Fig. 4. Quantitative analysis revealed that RME significantly increased early

(Fig. 5A) and total apoptotic cell percentages (Fig. 5C) compared to controls at concentrations of 100 and 200 µg/mL ($p < 0.001$). Importantly, the late apoptotic fraction (Fig. 5B) was significantly elevated at all doses (50–200 µg/mL) relative to control ($p < 0.001$).

AgNOR Analysis

The antiproliferative effect of RME was examined by AgNOR staining. Mean AgNOR number and the total AgNOR area per nuclear area (TAA/NA) for each treatment group are summarized in Table 1. No significant difference was observed between the control and 50 µg/mL RME group ($p = 0.073$). However, both mean AgNOR number and TAA/NA ratio were significantly reduced at 100 and 200 µg/mL compared with control ($p < 0.001$). Pairwise comparisons confirmed significant differences between 50 µg/mL and both 100 and 200 µg/mL RME groups ($p < 0.001$), indicating a clear dose-dependent antiproliferative effect (Table 2).

Table 1
TAA/NA and mean AgNOR number values of controls, 50 µg/mL RME, 100 µg/mL RME and 200 µg/mL RME treated MDA-MB-231 cells groups.

Groups	TAA/NA (Mean ± SD)	Mean AgNOR number (Mean ± SD)
Control-1 (n = 50)	0.104 ± 0.042	2.845 ± 1.282
Control-2 (n = 50)	0.101 ± 0.081	2.841 ± 1.209
Control-3 (n = 50)	0.105 ± 0.063	2.848 ± 0.863
50 µg/mL RME − 1 (n = 50)	0.102 ± 0.102	2.739 ± 1.099
50 µg/mL RME − 2 (n = 50)	0.99 ± 0.086	2.767 ± 1.069
50 µg/mL RME-3 (n = 50)	0.103 ± 0.075	2.775 ± 1.119
100 µg/mL RME 1 (n = 50)	0.92 ± 0.045	1.879 ± 1.289
100 µg/mL RME 2 (n = 50)	0.96 ± 0.062	1.893 ± 1.109
100 µg/mL RME 3 (n = 50)	0.93 ± 0.101	1.882 ± 1.198
200 µg/mL RME 1 (n = 50)	0.95 ± 0.019	1.812 ± 1.114
200 µg/mL RME 2 (n = 50)	0.91 ± 0.108	1.789 ± 1.102
200 µg/mL RME 3 (n = 50)	0.89 ± 0.082	1.829 ± 1.132
TAA/NA: Total AgNOR area/nuclear area, n: Number of measurements in each group.		

Table 2
Pairwise comparisons of mean AgNOR number and TAA/NA ratio in control and RME-treated MDA-MB-231 cells.

Comparison Groups	TAA/NA (Mean ± SD)	Mean AgNOR number (Mean ± SD)	p (TAA/NA)	p (AgNOR)
Control vs. 50 µg/mL RME	0.310 ± 0.003 vs 0.304 ± 0.001	8.534 ± 0.201 vs 8.281 ± 0.081	0.073	0.109
Control vs. 100 µg/mL RME	0.310 ± 0.003 vs 0.281 ± 0.005	8.534 ± 0.201 vs 5.654 ± 0.262	0.001*	< 0.001*
Control vs. 200 µg/mL RME	0.310 ± 0.003 vs 0.275 ± 0.003	8.534 ± 0.201 vs 5.430 ± 0.239	< 0.001*	< 0.001*
50 µg/mL vs. 100 µg/mL RME	0.304 ± 0.001 vs 0.281 ± 0.005	8.281 ± 0.081 vs 5.654 ± 0.262	0.001*	< 0.001*
50 µg/mL vs. 200 µg/mL RME	0.304 ± 0.001 vs 0.275 ± 0.003	8.281 ± 0.081 vs 5.430 ± 0.239	< 0.001*	< 0.001*
100 µg/mL vs. 200 µg/mL RME	0.281 ± 0.005 vs 0.275 ± 0.003	5.654 ± 0.262 vs 5.430 ± 0.239	0.223	0.253

Values are presented as mean ± standard deviation (SD). Statistical analysis was performed using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. *p < 0.05 was considered statistically significant. TAA/NA: Total AgNOR area per nuclear area. n = 50 nuclei per group.

DISCUSSION

This study investigated the effects of Raphanus sativus methanolic extract (RME) on MDA-MB-231, a highly aggressive triple-negative breast cancer cell line, and demonstrated a significant reduction in cell viability, increased apoptosis, and suppression of proliferative activity as confirmed by AgNOR analysis. These findings support the potential anticancer properties of Raphanus sativus and indicate that natural phytochemicals may play a supportive role in cancer management.

Nutrition is increasingly recognized as a modifiable factor that can influence cancer prevention, progression, and treatment outcomes. Natural bioactive compounds derived from vegetables, fruits, and spices exert a variety of biological effects, including antioxidant, antiproliferative, anti-inflammatory, and antitumoral mechanisms [25]. Several plant-based agents have been reported to selectively induce cytotoxicity in malignant cells while sparing healthy cells, making them attractive candidates as adjunctive therapeutic options. Herbal products have long been used in the management of chronic diseases due to their relative safety, cost-effectiveness, and long-term beneficial effects. Their anticancer effects are largely thought to occur through the regulation of the cell cycle, inhibition of signaling pathways, and induction of autophagy and apoptosis [26]. However, challenges such as bioavailability and optimal dosing remain important limitations [27].

Members of the cruciferous family, including broccoli, cabbage, and cauliflower, are rich in glucosinolates, whose hydrolysis products, isothiocyanates, have been linked to cancer-preventive effects [28]. Isothiocyanates have been shown in various cancer models to regulate detoxification enzymes, induce apoptosis, and inhibit cell cycle progression [29–31]. In line with these studies, our findings demonstrated that RME decreased cell viability in MDA-MB-231 cells in a dose-dependent manner, with an IC50 value of 140.5 µg/mL, and significantly increased apoptosis rates as revealed by Annexin V staining. Previous studies have similarly reported that radish extracts exert apoptotic effects in oral squamous carcinoma and other epithelial tumor cell lines via β-catenin suppression and caspase-3 activation [32,33].

AgNOR staining further confirmed the antiproliferative effects of RME in this study. The reduction in mean AgNOR count and TAA/NA ratios following increasing concentrations of RME reflects decreased ribosomal biogenesis and proliferative activity. Considering that AgNOR parameters are regarded as reliable indicators of cellular metabolic activity and protein synthesis capacity [34], these findings further support the antitumoral potential of RME. The most consistent effects were observed at concentrations of 100 µg/mL and 200 µg/mL, suggesting that these doses may represent biologically active ranges.

Translational Implications for Physiotherapy-Based Cancer Rehabilitation

Although this study focused on cellular endpoints, the observed findings conceptually align with the physiological targets of physiotherapy-based cancer rehabilitation. Chronic fatigue is one of the most common and burdensome complications in cancer patients [35]. Its underlying mechanisms include cancer-related systemic inflammation, infiltration of inflammatory markers into muscle tissue, and the consequent increase in protein degradation. These processes contribute to heightened fatigue perception, reduced exercise capacity, and diminished quality of life [36].

The induction of apoptosis observed in our study may contribute to the reduction of systemic inflammation and metabolic stress. This, in turn, could indirectly alleviate fatigue perception, positively influence muscle metabolism, and enhance exercise tolerance [37]. Similarly, the suppression of proliferative activity may help mitigate cancer-related cachexia and disturbances in protein turnover, thereby supporting muscle strength preservation and functional independence [38].

Furthermore, phytochemicals derived from radish have been reported to reduce oxidative stress and prevent mitochondrial dysfunction [32,33]. Such effects parallel those of physiotherapy interventions, including aerobic and resistance exercise, which are known to enhance mitochondrial efficiency and decrease oxidative damage [39]. Therefore, phytotherapy and physiotherapy may act synergistically to improve endurance, functional capacity, and quality of life in cancer survivors.

Limitations

This study has several limitations. First, it was conducted exclusively in vitro on a single aggressive breast cancer cell line (MDA-MB-231), which limits generalizability. Second, physiological parameters specific to physiotherapy—such as oxidative stress, inflammation, mitochondrial function, muscle strength, or exercise capacity—were not directly measured, and links to physiotherapy remain conceptual. Third, the molecular pathways underlying the effects of RME (e.g., caspase activation, mitochondrial apoptotic signaling, ROS levels) were not examined in detail. Finally, the study employed only a short-term (24-hour) exposure, preventing evaluation of longer-term or time-dependent effects.

Future Directions

Future studies should confirm the apoptotic and antiproliferative effects of RME in other breast cancer subtypes and in vivo models. Detailed pathway analyses (e.g., caspases, Bcl-2 family proteins, mitochondrial membrane potential, ROS production) are required to clarify mechanisms. From a translational perspective, research should also explore combining RME with physiotherapy-based rehabilitation programs, with a focus on exercise capacity, muscle strength, fatigue, quality of life, and oxidative stress parameters in cancer patients. Furthermore, dosage optimization, safety, and bioavailability must be addressed for clinical translation.

CONCLUSION

This study demonstrated that Raphanus sativus methanolic extract (RME) exerts significant cytotoxic, apoptotic, and antiproliferative effects in MDA-MB-231 triple-negative breast cancer cells, as evidenced by MTT, Annexin V, and AgNOR analyses. To our knowledge, this is the first study to simultaneously evaluate these outcomes in this cell line using these three complementary methods. Beyond its basic oncological implications, the originality of this work lies in contextualizing the findings within the framework of physiotherapy-based oncological rehabilitation. Although the results are derived solely from an in vitro model and must be interpreted with caution, they conceptually align with the physiological targets of cancer rehabilitation and suggest that phytotherapy and physiotherapy could act synergistically. Thus, this study provides preliminary translational evidence and a novel interdisciplinary perspective, bridging basic science with rehabilitation practice and offering a foundation for future research on integrated cancer management strategies.

Declarations

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Ethical Approval

Ethical approval is not required for cell line studies

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Disclosure statement

No potential conflict of interest was reported by the authors.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

Author contribution

The authors confirm their contribution to the paper as follows: study conception and design: M.N., U.S., F.D., N.İ., G.B.U.; data collection: M.N., F.D.; analysis and interpretation of results: M.N., U.S., F.D., N.İ., GBU; draft manuscript preparation: M.N., U.S., F.D., N.İ., GBU All authors reviewed the results and approved the final version of the manuscript.

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Figures

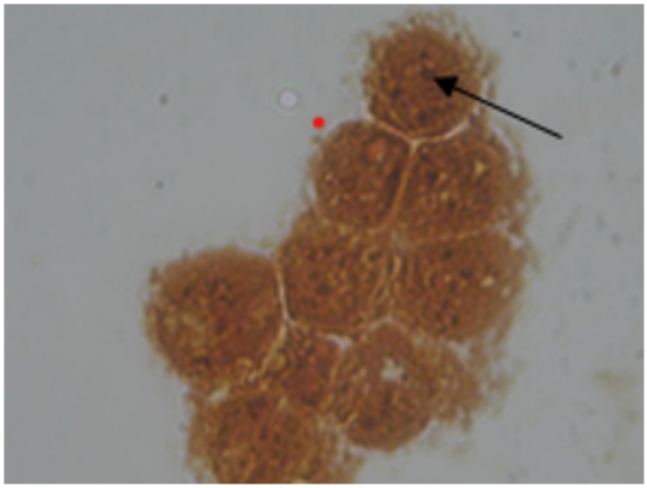

Figure 1

A demonstrative example of AgNOR staining of MDA-MB-231 cells (×20 magnification, Scale bar 200 mm, arrows shows argyrophilic nucleolar protein). AgNOR; argyrophilic nucleolar organizing region-associated protein.

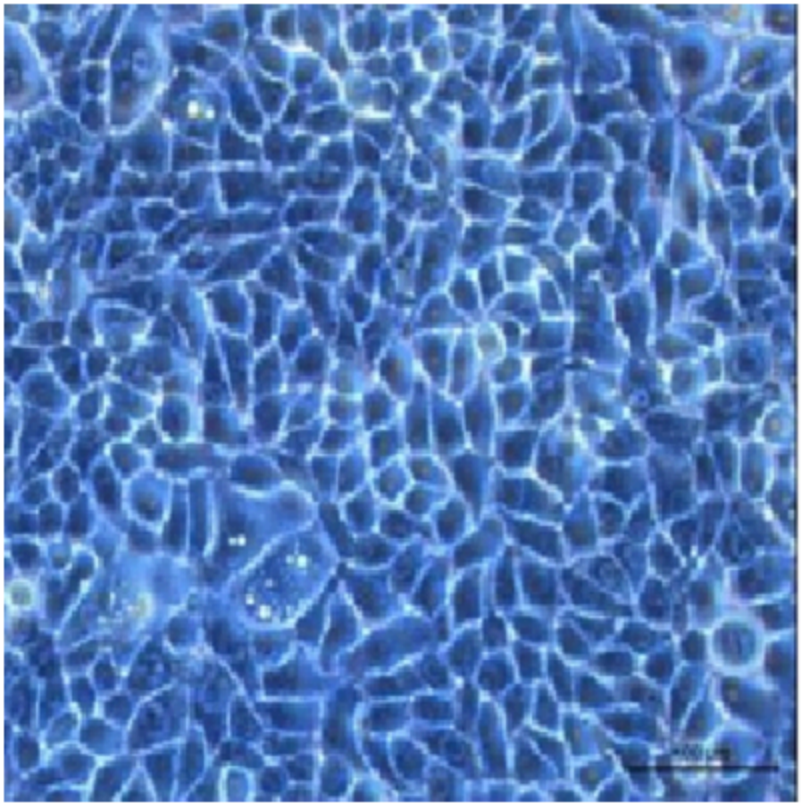

Figure 2

MDA-MB-231 cell line (20X)

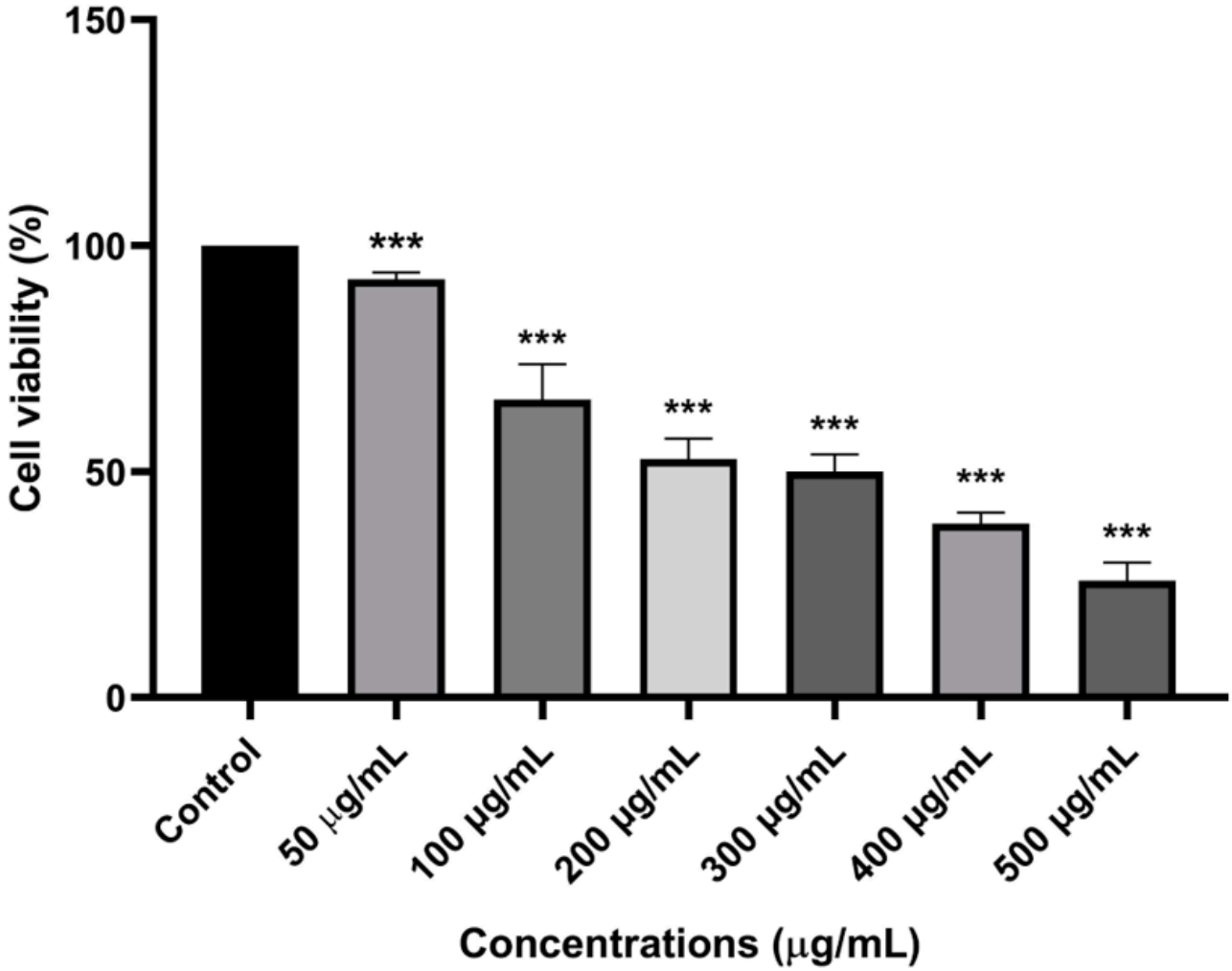

Figure 3

Effect of RME on cell viability in MDA-MB-231 cells

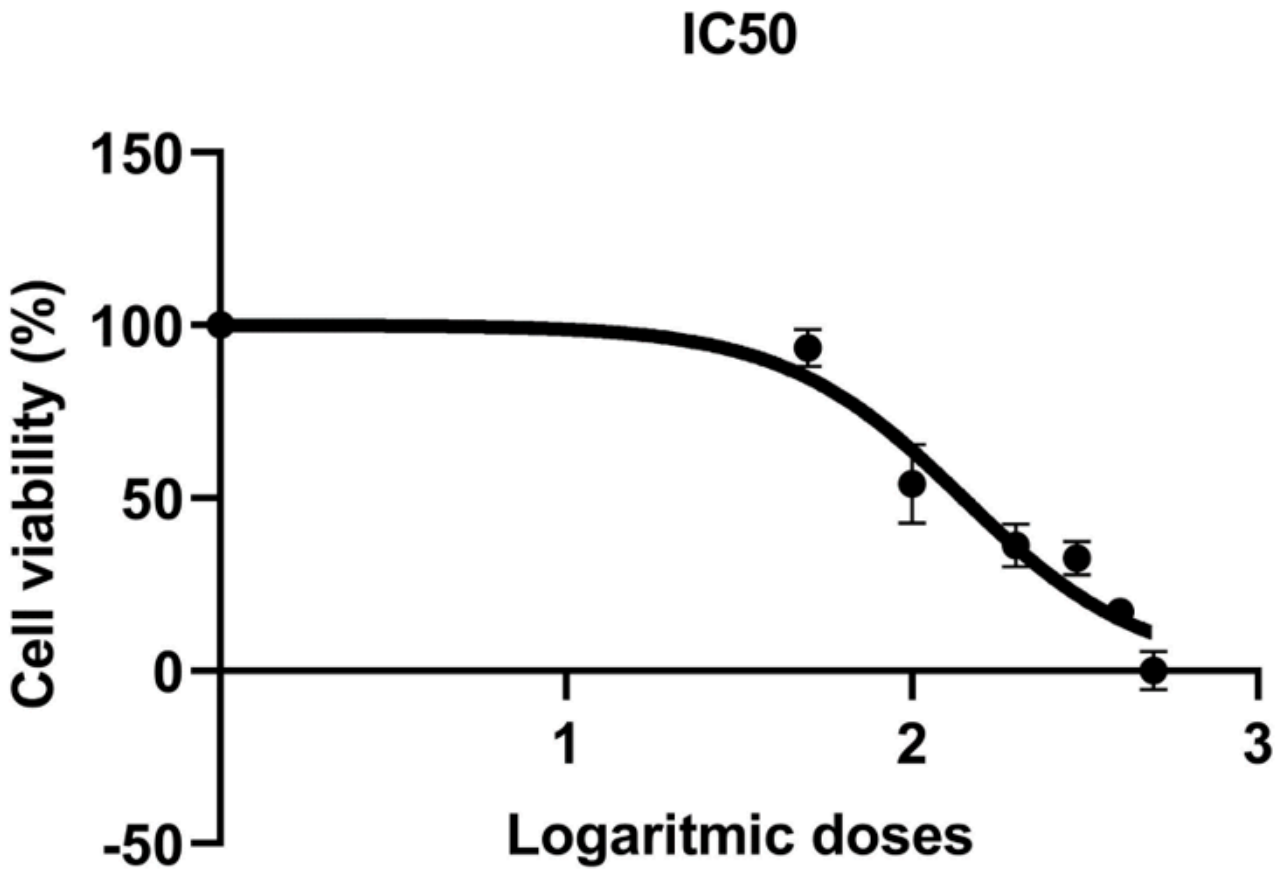

Figure 4

IC50 dose of RME in MDA-MB-231 cells

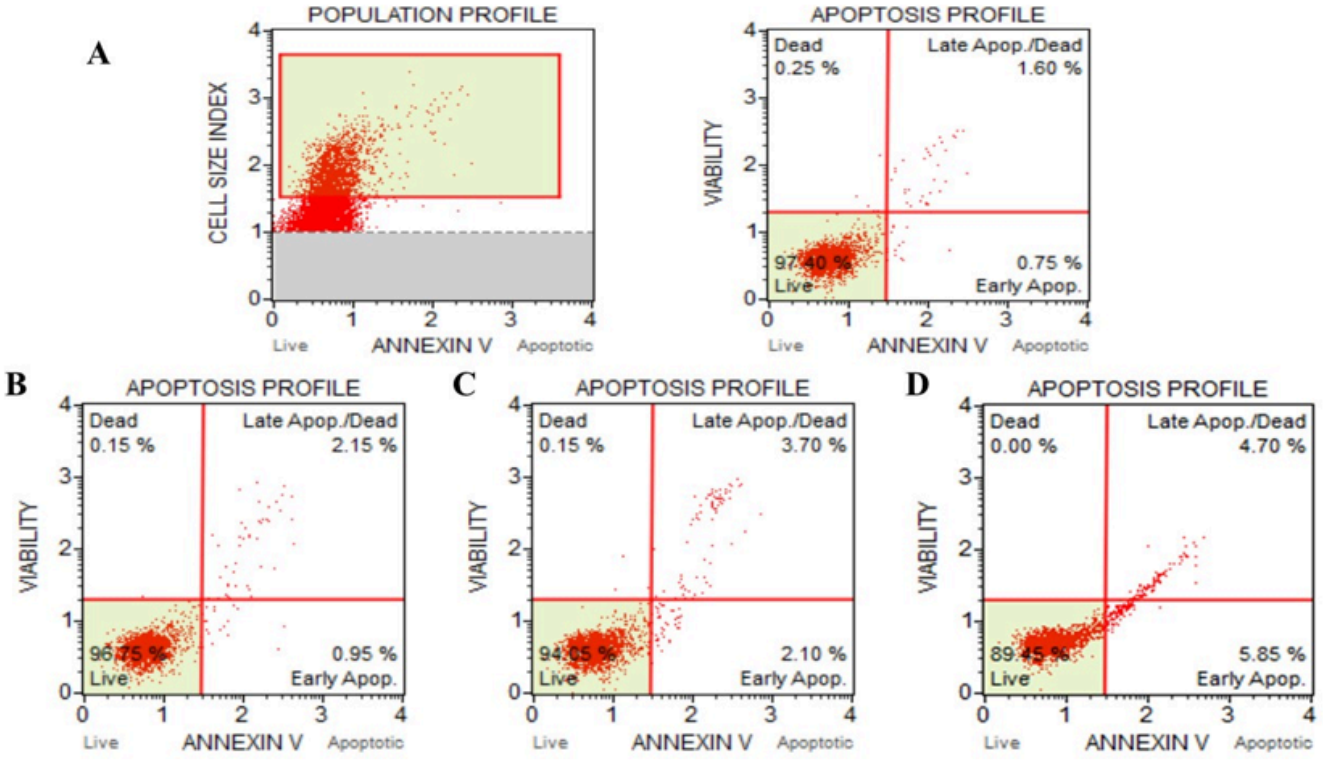

Figure 5

Demonstration of the effects of RME on apoptosis in MDA-MB-231 cells by Annexin V staining analysis, A: Control, B: 50 µg/mL, C: 100 µg/mL, D: 200 µg/mL

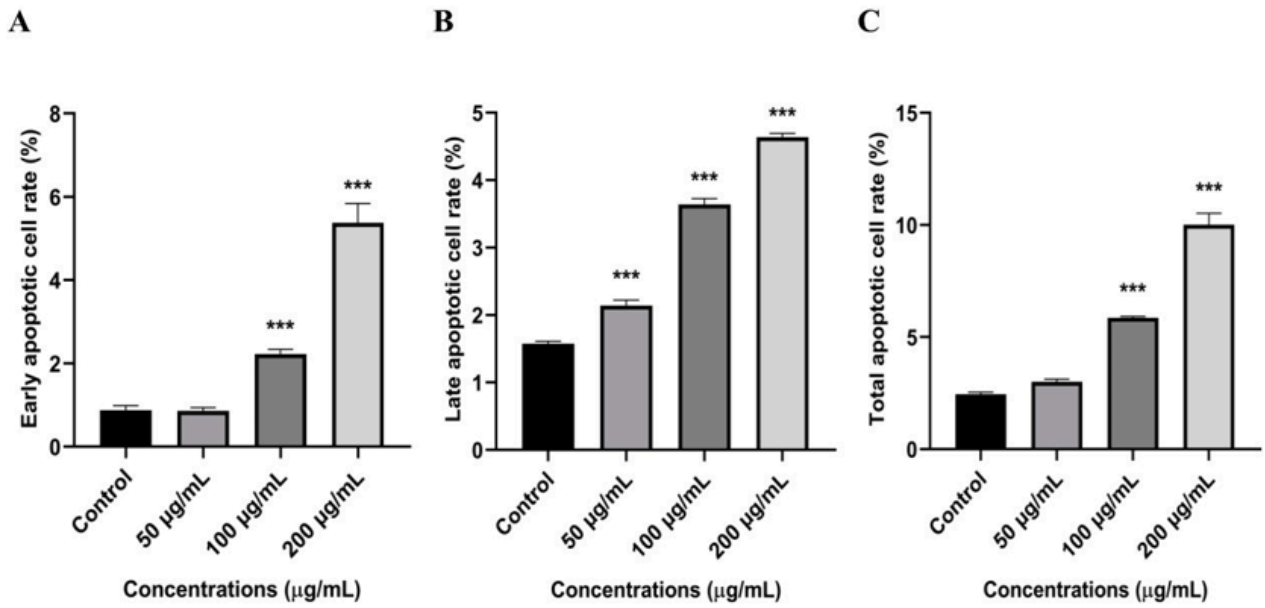

Figure 6

Effect of RME on apoptosis in MDA-MB-231 aggressive breast cancer cell line, A: Early apoptosis, B: Late apoptosis, C: Total apoptosis