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Cytotoxic and Pro-Apoptotic Effects of Panax Ginseng on MDA-MB-231 Breast Cancer Cells

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ABSTRACT

Breast cancer remains the most prevalent malignancy among women globally and continues to be a primary contributor to cancer-associated mortality due to its high metastatic potential and resistance to conventional therapies. In light of this clinical significance, the present study aimed to elucidate the cytotoxic and pro-apoptotic properties of *Panax ginseng* extract on the triple-negative breast cancer cell line MDA-MB-231.

Cells were exposed to a range of ginseng concentrations, and cytotoxic effects were quantified via the MTT assay. The half-maximal inhibitory concentration (IC_{50}) of ginseng was established at 226.50 µg/mL. Immunocytochemical evaluations at the IC_{50} concentration were conducted to assess apoptotic induction and DNA damage through detection of caspase-3 and PARP-1 (poly(ADP-ribose) polymerase 1), expression.

Treatment with ginseng elicited a concentration-dependent reduction in cellular viability. At the IC_{50} dose, upregulation of caspase-3 and PARP-1 was noted, indicative of apoptotic pathway activation. Moreover, alterations in nuclear morphology and immunostaining patterns suggested the occurrence of DNA damage in ginseng-exposed cells.

Collectively, these data demonstrate that ginseng mediates cytotoxicity in MDA-MB-231 cells via apoptosis induction and DNA damage, underscoring its therapeutic potential against triple-negative breast cancer.

Keywords: Apoptosis, caspase-3, MDA-MB-231, *Panax Ginseng,* PARP-1.

Introduction

Breast cancer constitutes the most prevalent malignancy among women globally and remains a primary contributor to cancer-associated mortality (1). Given its molecular heterogeneity, breast cancer is stratified into distinct subtypes based on the expression profiles of key receptors. The MDA-MB-231 cell line, a well-established *in vitro* model of triple-negative–breast cancer (TNBC), is defined by the absence of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2. This subtype is notably characterized by its highly invasive phenotype, rapid proliferative capacity, and pronounced metastatic potential (2).

Panax ginseng, extensively utilized in traditional East Asian therapeutics, has been reported to exert diverse pharmacodynamic actions, including free radical scavenging, modulation of inflammatory pathways, and inhibition of tumorigenesis (3-5). These effects are largely attributed to its bioactive triterpene saponins, known as ginsenosides (e.g., protopanaxadiol [PPD], Rg3, Rg5, Rh1, and Rh2), which have been reported to inhibit cancer cell proliferation, suppress metastasis, and importantly, induce apoptosis (6,7). While a recent study (8) investigated the pro-apoptotic and anti-EMT activities of wild ginseng root in relation to GSK-3β/β-catenin signaling in MDA-MB-231 cells, there is still a need to further characterize the direct impact of standardized *Panax ginseng* extract on terminal apoptotic executioners.

Apoptosis, a form of programmed cell death, is essential for the removal of damaged or abnormal cells and is often dysregulated in cancer. Caspase-3 is a key executioner protease in this process, mediating the cleavage of cellular substrates and ultimately driving apoptotic cell death (9). Recent

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studies demonstrated that ginsenosides trigger apoptosis in breast cancer cells via caspase-dependent mechanisms. Specifically, in MDA-MB-231 cells, ginsenoside Rg3 treatment significantly increased levels of cleaved caspase-3 and PARP-1, indicating activation of the caspase-mediated apoptotic pathway (10). Similarly, ginsenoside Rh1 has been shown to induce mitochondrial apoptosis through reactive oxygen species (ROS) generation and caspase-3 activation in the same cell line (11).

In light of these findings and to address the remaining gaps in understanding the terminal phase of ginseng-induced death, the present study aimed to investigate the cytotoxic effects of *Panax ginseng* at its IC_{50} concentration on MDA-MB-231 cells. Furthermore, we employed immunocytochemical analysis to specifically assess the expression of caspase-3 and PARP-1, providing a targeted insight into the executioner mechanisms and DNA damage response triggered by this treatment, which remains a critical area of investigation in TNBC therapy.

Materials and Methods

Preparation of *Panax Ginseng:* *Panax ginseng* extract was purchased from Sigma-Aldrich (St. Louis, MO, USA; Cat No: 05115001). 0.0623g of the extract was accurately weighed and dissolved in 1mL of dimethylsulfoxide (DMSO; Sigma-Aldrich) to prepare a 62.3 mg/mL stock solution. The solution was freshly prepared, sterilized with a 0.22 µm syringe filter, and stored at −20°C until use. The final concentration of DMSO in the treatment groups was maintained below 0.5% (v/v) to ensure no solvent-induced cytotoxicity.

Cell Propagation and Maintenance: The human breast cancer cell line MDA-MB-231 which was bought from ATCC was utilized as the experimental model. The cells were cultured in Dulbecco's Modified Eagle's Medium-high glucose (DMEM-hg; Sigma-Aldrich, D6429), supplemented with 10% fetal bovine serum (FBS; Gibco, 10270106) and 1% GlutaMAX (Gibco, 35050061). Cultures were maintained in a humidified incubator at 37°C with 5% CO_2.

When the cells reached approximately 85–90% confluency, the culture medium was aspirated, and the cell monolayer was rinsed twice with phosphate-buffered saline (PBS) to remove residual medium. Subsequently, cells were detached by incubation with 0.25% Trypsin-EDTA (Sigma-Aldrich, T4049) for 5 minutes at 37°C. The enzymatic reaction was neutralized by adding 3 mL of complete growth medium, and the cell suspension was transferred to a 15 mL centrifuge tube. The suspension was then centrifuged at 300 × g for 10 minutes.

Following centrifugation, the supernatant was carefully discarded, and the cell pellet was resuspended in 5 mL of fresh medium. Cell counting and viability assessment were performed using a Thoma counting chamber via the trypan blue (0.4%) exclusion method to ensure high cellular viability prior to seeding.

***In vitro* Antiproliferative Activity Studies:** The cytotoxic activity of *Panax ginseng* was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, following standardized protocols (12). MDA-MB-231 cells were seeded into sterile 96-well microplates at a density of 5×103 cells per well and incubated for 24 hours at 37°C to ensure adequate adhesion.

Following the incubation period, the culture medium was replaced with 100µL of fresh medium. To determine the dose-dependent inhibitory trend and establish the concentration range for further analysis, cells were exposed to *Panax ginseng* extract as a preliminary screening at standardized concentrations of 311.5, 155.75, 77.875, 38.9375, and 19.46 µg/mL for a duration of 48 hours. A vehicle control group containing the same maximum concentration of DMSO (below 0.5% v/v) used in the treatment groups was included in the experimental design to account for any potential solvent-induced cytotoxicity.

Upon completion of the treatment period, the medium was aspirated, and 20µL of MTT stock solution (5mg/mL) was added to each well. The plates were incubated for an additional 2 hours at 37°C to allow for mitochondrial reduction of MTT into formazan crystals. Subsequently, the supernatant was gently removed under light-protected conditions, and the resulting crystals were solubilized using 200µL of DMSO. The plates were agitated in the dark at ambient temperature for 30 minutes.

Absorbance was measured at 570 nm using an Agilent BioTek microplate reader. The percentage of cell viability was calculated relative to the vehicle control. The half-maximal inhibitory concentration (IC_{50}) value was estimated by nonlinear regression analysis (sigmoidal dose-

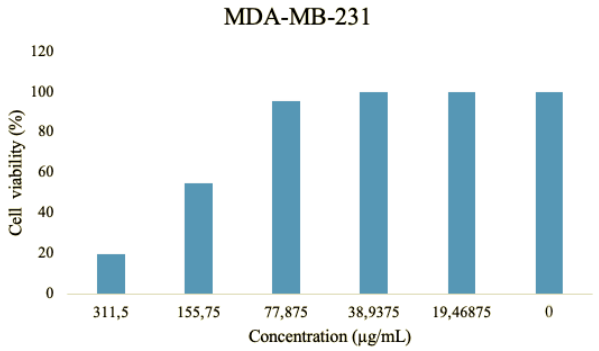

Fig. 1. The cell viability ratio depending on various concentrations of *Panax Ginseng*

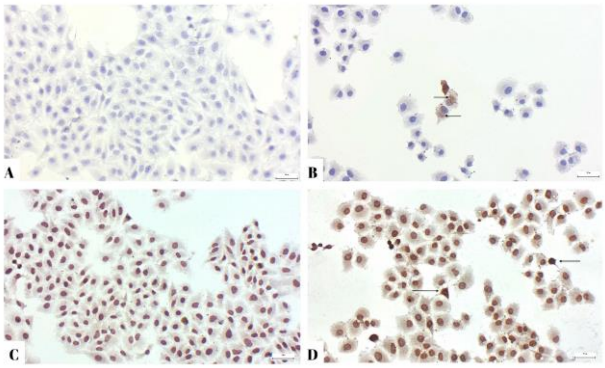

Fig. 2. Cells were treated with the calculated IC_{50} concentration of *Panax ginseng* extract (226.50 µg/mL) for 48 hours to evaluate its pro-apoptotic potential. (A) Untreated control group for Caspase-3, showing negligible baseline immunoreactivity. (B) *Panax ginseng*-treated group showing marked cytoplasmic Caspase-3 expression. (C) Untreated control group for PARP-1, exhibiting no significant staining. (D) *Panax ginseng*-treated group demonstrating prominent nuclear PARP-1 immunoreactivity. Positive brown DAB staining (indicated by black arrows) confirms the upregulation of these apoptotic markers in response to ginseng treatment compared to the respective control groups. All images were captured at 400x magnification; Scale bar = 50 µm. Images are representative of three independent biological observations

response) using GraphPad Prism 5 software based on the representative screening data.

Immunocytochemistry: The expression levels of Caspase-3 and PARP-1 were evaluated using immunocytochemical staining to assess the pro-apoptotic effects of *Panax ginseng* treatment. MDA-MB-231 cells were seeded onto sterile coverslips in 6-well plates and treated with *Panax ginseng* at the determined IC_{50} concentration of 226.50 µg/mL for 48 hours. Following the treatment period, cells were fixed with 4% paraformaldehyde for 15 minutes at room temperature and permeabilized with 0.1% Triton X-100 in phosphate-buffered saline (PBS).

Endogenous peroxidase activity was quenched by incubating the cells in 3% hydrogen peroxide (H2 O2) for 10 minutes. To prevent non-specific binding, cells were blocked with 5% normal goat serum for 30 minutes. The cells were then incubated overnight at 4°C with the following primary antibodies: rabbit polyclonal anti-Caspase-3 (Cell Signaling Technology, Cat No: 9661, 1:400 dilution) and rabbit polyclonal anti-PARP-1 (Proteintech, Cat No: 13371-1-AP, 1:200 dilution).

After washing with PBS, the sections were incubated with a ready-to-use HRP-conjugated horse anti-rabbit IgG polymer detection kit (ImmPRESS®; Vector Laboratories, Burlingame, CA, USA; Cat No: MP-7601) for 30 minutes at room temperature. The immunoreactivity was visualized using 3,3'-diaminobenzidine (DAB) chromogen, and the cells were counterstained with Mayer's hematoxylin. Negative control groups, in which the primary antibodies were replaced with PBS, were included in each staining run to verify the specificity of the immunoreactivity.

The stained coverslips were examined under a light microscope (Zeiss Axioscope 5, Germany), and a qualitative microscopic evaluation was performed by assessing the distribution and intensity of the brown DAB staining across five random fields per group. The protocol was conducted in accordance with established immunocytochemical procedures.

Results

Antiproliferative Activity of *Panax Ginseng* on MDA-MB-231 Cells: The cytotoxic effects of *Panax ginseng* on MDA-MB-231 cells were evaluated using a cell viability assay across a range of concentrations (311.5, 155.75, 77.875, 38.9375, and 19.46875 µg/mL). The data revealed a dose-dependent decrease in cell viability with increasing concentrations of *Panax ginseng*. At the highest concentration (311.5 µg/mL), cell viability was significantly reduced, reaching approximately 20% of the control group. In contrast, lower concentrations (19.46875 µg/mL and 38.9375 µg/mL) showed viability levels near 80-90%, indicating that *Panax ginseng* induces cytotoxicity in MDA-MB-231 cells in a concentration-dependent manner. The calculated IC_{50} value for MDA-MB-231 cells was found to be 226.50 µg/mL (Table 1), confirming the potency of *Panax ginseng* in inhibiting cell viability. These results suggest that *Panax ginseng* exhibits potent inhibitory effects on the viability of MDA-

Table 1: IC_{50} Result For Ginseng in MDA-MB-231 Cell Line

Compound	IC_{50} (µg/mL) MDA-MB-231
Ginseng	226.50

MB-231 cells, particularly at higher concentrations (Figure 1).

Immunocytochemical Analysis of Caspase-3 and PARP-1 in MDA-MB-231 Cells: Immunocytochemical analysis was performed to qualitatively evaluate the expression levels of Caspase-3 and PARP-1 in MDA-MB-231 cells following treatment with *Panax ginseng* at the IC_{50} dose (226.50 µg/mL). Microscopic examination revealed a marked and observable increase in the immunoreactivity of both apoptotic markers in the treatment groups compared to the untreated control cells (Figure 2).

Cells treated with *Panax ginseng* exhibited intense and widespread brown cytoplasmic DAB staining for Caspase-3, indicating a clear upregulation of this executioner protease. Similarly, a prominent increase in nuclear staining for PARP-1 was observed, suggesting the activation of DNA damage response mechanisms and apoptotic commitment. In contrast, the control group showed negligible or no staining for either marker (Figure 2). These representative qualitative observations, characterized by distinct alterations in staining intensity and cellular morphology, provide evidence that *Panax ginseng* effectively triggers the apoptotic cascade in MDA-MB-231 cells.

Discussion

In this study, the cytotoxic effects of *Panax ginseng* extract on the MDA-MB-231 breast cancer cell line were evaluated, and the IC_{50} value was determined to assess dose-dependent inhibition of cell proliferation. Furthermore, the pro-apoptotic effect of *Panax ginseng* was investigated in MDA-MB-231 cells via immunocytochemical detection of Caspase-3 and PARP-1, both critical mediators in the apoptotic cascade.

Our findings indicate that *Panax ginseng* exerts a dose-dependent cytotoxic effect on MDA-MB-231 cells, with an IC_{50} value of 226.50 µg/mL, indicating its potent inhibitory effect on cell viability. This value is consistent with the bioactive potential of ginsenosides reported in various TNBC models. The increased sensitivity of MDA-MB-231 cells to *Panax ginseng* may be attributed to their more aggressive and metabolically active phenotype, making them more susceptible to stress induced by natural compounds (13). A very recent study by Hong et al. (8) demonstrated that wild ginseng root extract suppresses the epithelial-mesenchymal transition (EMT) in MDA-MB-231 cells via the GSK-3β/β-catenin signaling pathway. While their work emphasizes signaling modulation, our findings complement this by providing evidence for the activation of the terminal apoptotic phase.

The balance between pro-apoptotic and anti-apoptotic proteins is a key factor in determining a cell's sensitivity or resistance to apoptotic signals (14). Upon mitochondrial release, apoptotic mediators can activate Caspase-3, a crucial enzyme involved in the execution phase of apoptosis (15, 16). The increased expression of Caspase-3 observed in MDA-MB-231 cells after treatment with *Panax ginseng* extract confirms the activation of the apoptotic pathway. Additionally, increased PARP-1 expression was observed, supporting the involvement of DNA repair and apoptosis activation in the treatment response.

The observed upregulation of PARP-1 immunoreactivity reflects a complex cellular response to ginseng-induced genomic stress. Recent research reported that ginsenosides can induce oxidative DNA damage (25), which significantly exhausts the DNA repair machinery. The prominent increase in PARP-1 staining likely indicates an active DNA damage response (DDR) prior to the final commitment to cell death, a mechanism also supported by Zhao and Wang (26) in standardized ginseng models. During apoptosis, PARP-1 undergoes cleavage by Caspase-3, leading to the inhibition of its DNA repair function and the promotion of cell death (17, 18). The activation of PARP-1 and its subsequent cleavage are often considered hallmarks of both apoptosis and DNA damage responses, further confirming the involvement of both pathways in the action of *Panax ginseng*. These findings align with previous studies that have reported ginsenosides can trigger mitochondrial-mediated apoptosis through the activation of Caspase-3 and PARP-1 (10, 19).

Panax ginseng exhibits a broad spectrum of pharmacological activities, including anticancer,

antioxidant, and anti-inflammatory effects (20-22). Specifically, ginsenosides such as Rg3 and Rh2 have shown potent antiproliferative effects in cancer models (23). These findings support the results of our study, suggesting that *Panax ginseng* may exert its cytotoxic effects through modulation of apoptotic signaling in TNBC cells.

Although our findings provide valuable insights into the anticancer potential of *Panax ginseng*, several limitations must be acknowledged. The chemical composition of the extract was not fully characterized, which limits the ability to attribute the observed effects to specific ginsenosides. Furthermore, since this research utilized a single cell line, the potential for a "particularly effective" action against all TNBC subtypes remains to be validated with further comparative studies including non-cancerous control lines. Moreover, apoptosis was assessed primarily by Caspase-3 and PARP-1 immunocytochemistry. It should be noted that our analysis targeted total protein expression; the lack of Western blot or Annexin V/PI flow cytometry to specifically distinguish between total and cleaved protein forms represents a methodological limitation. Additionally, complementary analyses such as mitochondrial membrane potential assays or synergy studies (24) would provide a more comprehensive understanding of the underlying molecular mechanisms.

In conclusion, this study demonstrates that *Panax ginseng* exerts significant cytotoxic and pro-apoptotic effects on the MDA-MB-231 human breast cancer cell line. The determination of the IC_{50} value confirmed dose-dependent cytotoxicity, with MDA-MB-231 cells showing notable sensitivity to the ginseng extract. Furthermore, increased Caspase-3 and PARP-1 expression, observed through immunocytochemical analysis, indicates that the activation of the apoptotic cascade is a key mechanism underlying the antiproliferative activity of *Panax ginseng*.

Considering the purely *in vitro* nature of these findings, they should be regarded as preliminary. While the results underscore the potential of *Panax ginseng* as a candidate for further investigation in the management of triple-negative breast cancer (TNBC), its role as a complementary therapeutic agent requires validation through more comprehensive studies. Specifically, the involvement of Caspase-3 and PARP-1-mediated pathways provides a foundational understanding that warrants further *in vivo* studies and detailed molecular analyses to confirm the clinical relevance and safety of these observations.

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