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ANTICANCER ACTIVITY OF EPIGALLOCATECHIN GALLATE AGAINST MDA-MB-231 TRIPLE-NEGATIVE BREAST CANCER CELLS THROUGH OXIDATIVE STRESS-INDUCED APOPTOSIS

Jamunarani Srirangaramasamy[1], *Sasikumar Arumugam[2], Vishnupriya J [3] Sivanesan Senthilkumar[4] Radhika M [5]

[1] Research Scholar & Professor & Head, Department of Pathology BMCH, BIHER, Meenakshi Academy of Higher Education and Research, MAHER, KK Nagar, Chennai, India.
[*2] Professor, Department of Physiology, VELS Medical College and Hospital, VISTAS, Periyapalayam Campus, Tamil Nadu, India.
[3] Tutor, Department of Biochemistry, Panimalar college of Allied Health Sciences, Panimalar Medical College and HRI, Varadharajpuram, Poonamallee, Chennai. 600123, India.
[4] Associate Professor. CLAR-Animal House, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai,602105 Tamilnadu, India.
[5] Associate Professor, Department of Biochemistry, VELS School of Allied Health Sciences, VMCH, VISTAS, Periyapalayam Campus, Tamil Nadu, India.
*Corresponding Author: Dr Sasikumar, , Email: simmamsasi@gmail.com,

ABSTRACT

Background: Triple-negative breast cancer (TNBC) is an aggressive form of breast cancer characterized by the absence of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor-2 (HER2). Owing to the lack of specific molecular targets, conventional chemotherapy remains the principal treatment modality, although therapeutic outcomes are often limited by recurrence and drug resistance. Epigallocatechin-3-gallate (EGCG), the predominant polyphenolic catechin found in green tea, has shown considerable promise because of its ability to inhibit tumour growth through multiple molecular mechanisms.

Objective: To investigate the cytotoxic effect of EGCG on MDA-MB-231 triple-negative breast cancer cells and to elucidate its mechanism of action by assessing oxidative stress, apoptosis, and membrane damage.

Materials and Methods: MDA-MB-231 cells were exposed to EGCG at concentrations ranging from 0 to 50 μM for 24 hours. Cell viability was determined using a colorimetric cytotoxicity assay. Intracellular reactive oxygen species (ROS) levels were quantified using the DCFH-DA fluorescence assay, while activation of caspase-3/7 was measured using a fluorometric assay. Membrane integrity was evaluated by estimating lactate dehydrogenase (LDH) release. Data were expressed as mean ± standard deviation and analyzed using one-way analysis of variance followed by Tukey's post hoc test. A p-value <0.05 was considered statistically significant.

Results: Exposure to EGCG resulted in a marked concentration-dependent reduction in the viability of MDA-MB-231 cells. Growth inhibition increased progressively from 6.37% in untreated cells to 78.06% following treatment with 50 μM EGCG. Intracellular ROS generation increased substantially, with fluorescence intensity rising from 220.5 in the control group to 4491.5 at the highest concentration tested. Caspase-3/7 activity also increased markedly, indicating activation of the apoptotic pathway, while LDH release showed a parallel increase, reflecting progressive loss of membrane integrity. These findings collectively indicate that EGCG suppresses tumour cell proliferation by promoting oxidative stress-mediated apoptosis.

Conclusion: EGCG demonstrated potent anticancer activity against MDA-MB-231 triple-negative breast cancer cells by reducing cell viability and inducing oxidative stress, caspase-dependent apoptosis, and membrane damage in a concentration-dependent manner. These findings support the potential role of EGCG as a promising natural therapeutic candidate for TNBC. Further preclinical and in vivo studies are warranted to establish its therapeutic efficacy and clinical applicability.

KEYWORDS: Epigallocatechin-3-gallate; EGCG; Triple-negative breast cancer; MDA-MB-231; Reactive oxygen species; Apoptosis; Caspase-3/7; Lactate dehydrogenase.

INTRODUCTION

Breast cancer remains the most frequently diagnosed malignancy among women and is one of the leading causes of cancer-related mortality worldwide. According to the Global Cancer Statistics 2022, approximately 2.3 million new breast cancer cases and more than 660,000 deaths were reported globally. Triple-negative breast cancer (TNBC) represents nearly 15–20% of all breast cancer cases and is characterized by the absence of estrogen receptors (ER), progesterone receptors (PR), and human epidermal growth factor receptor-2 (HER2). Because these receptors are lacking, targeted endocrine and HER2-directed therapies are ineffective, leaving systemic chemotherapy as the principal treatment option. Despite advances in chemotherapy, TNBC is associated with rapid disease progression, early metastasis, frequent recurrence, and poor overall survival. These challenges emphasize the need for safer and more effective therapeutic strategies. (1–3)

In recent years, naturally occurring bioactive compounds have emerged as promising candidates for cancer therapy because of their ability to interfere with multiple signalling pathways while exhibiting relatively low toxicity toward normal cells. Among these compounds, epigallocatechin-3-gallate (EGCG), the predominant catechin found in green tea (*Camellia sinensis*), has been extensively investigated for its anticancer potential. Experimental studies have demonstrated that EGCG suppresses tumour growth by regulating several cellular pathways involved in proliferation, inflammation, angiogenesis, invasion, and apoptosis, including PI3K/Akt, MAPK, NF-κB, STAT3, and Wnt/β-catenin signalling. These diverse biological actions have generated considerable interest in EGCG as a potential therapeutic agent against a variety of human cancers, including breast cancer. (4–7)

The biological activity of EGCG appears to depend largely on its ability to influence intracellular redox balance. Reactive oxygen species (ROS) are generated during normal cellular metabolism and participate in signalling pathways that regulate proliferation, differentiation, and survival. Excessive accumulation of ROS, however, overwhelms the cellular antioxidant defence system and results in oxidative damage to nucleic acids, proteins, and membrane lipids. This oxidative imbalance promotes mitochondrial dysfunction and initiates apoptotic cell death. Although EGCG functions as an antioxidant under physiological conditions, evidence suggests that it behaves as a pro-oxidant in malignant cells by selectively elevating intracellular ROS levels beyond their tolerance threshold, thereby promoting cancer cell death while largely sparing normal tissues. (8–11)

Apoptosis is a highly regulated mechanism that eliminates damaged or transformed cells without provoking an inflammatory response. Activation of the executioner enzymes caspase-3 and caspase-7 represents a critical event in this process and leads to chromatin condensation, DNA fragmentation, membrane blebbing, and formation of apoptotic bodies. As apoptosis progresses, disruption of plasma membrane integrity results in the release of intracellular enzymes such as lactate dehydrogenase (LDH), a widely accepted marker of cytotoxicity. Consequently, simultaneous assessment of ROS production, caspase activation, and LDH leakage provides valuable information regarding the mechanisms through which anticancer agents induce tumour cell death. (12–15)

Fluorescence-based techniques provide additional evidence of apoptosis by enabling visualization of cellular and nuclear alterations. The DCFH-DA assay is routinely employed to quantify intracellular ROS production, whereas Acridine Orange/Ethidium Bromide (AO/EB) dual staining differentiates viable, apoptotic, and necrotic cells according to membrane integrity and nuclear morphology. Propidium iodide (PI) staining further identifies cells that have lost membrane integrity during late apoptosis or necrosis. When interpreted together with phase-contrast microscopy, these techniques offer a comprehensive assessment of apoptosis and treatment-induced morphological changes. (16–18)

Among the available experimental models, the human MDA-MB-231 cell line is widely recognized as a representative model of TNBC because of its highly invasive behaviour, metastatic potential, and resistance to conventional chemotherapy. Although several investigations have reported the antiproliferative effects of EGCG in breast cancer cells, relatively few studies have comprehensively examined its influence on oxidative stress, apoptosis, membrane integrity, and cellular morphology using multiple complementary biochemical and fluorescence-based assays. (6,13,19)

Therefore, the present study was designed to evaluate the anticancer activity of EGCG against MDA-MB-231 triple-negative breast cancer cells by assessing its effects on cell viability, intracellular ROS generation, caspase-3/7 activation, LDH leakage, and apoptosis using complementary biochemical and fluorescence-based techniques. A better understanding of these mechanisms may provide additional evidence supporting the development of EGCG as a potential therapeutic agent for the management of triple-negative breast cancer.

MATERIALS AND METHODS:

Cell Culture

The human triple-negative breast cancer cell line MDA-MB-231 was procured from a certified cell repository and maintained under standard laboratory conditions. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin. Cell cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2. The culture medium was replaced at regular intervals, and cells were subcultured upon reaching approximately 80–90% confluence. Only healthy, exponentially growing cells were selected for subsequent experiments.

Experimental Design

To investigate the effect of EGCG on TNBC cells, MDA-MB-231 cells were seeded at an appropriate density in culture plates and allowed to attach overnight. Following attachment, the cells were exposed to 0, 10, 20, 30, 40, and 50 µM EGCG for 24 hours, while untreated cells served as the control group. After the incubation period, the effects of EGCG on cell viability, intracellular ROS generation, caspase-3/7 activation, and membrane integrity were evaluated using established biochemical assays.

Cell Viability Assay

Cell viability following EGCG treatment was assessed using a commercially available colorimetric cytotoxicity assay. Cells were seeded into 96-well microplates and incubated overnight to ensure adequate attachment. After exposure to the designated concentrations of EGCG for 24 hours, the assay reagent was added according to the manufacturer's instructions. Following incubation, absorbance was measured using a microplate reader. Cell viability was calculated relative to the untreated control, and the percentage of growth inhibition was determined for each treatment concentration.

Measurement of Intracellular Reactive Oxygen Species

Intracellular oxidative stress was quantified using the 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) fluorescence assay. Following EGCG treatment, the cells were washed gently with phosphate-buffered saline to remove residual culture medium and incubated with DCFH-DA under light-protected conditions. Fluorescence intensity was subsequently measured using a fluorescence microplate reader. Representative fluorescence images were also obtained using a fluorescence microscope. The results were expressed as mean fluorescence intensity (MFI).

Caspase-3/7 Activity Assay

Activation of apoptosis was evaluated by estimating caspase-3/7 activity using a fluorometric assay kit. After treatment with EGCG, the fluorogenic substrate supplied with the kit was added to each well according to the manufacturer's protocol. Fluorescence intensity was recorded using a fluorescence microplate reader, and caspase-3/7 activity was expressed as mean fluorescence intensity, reflecting the degree of apoptotic activation.

Lactate Dehydrogenase (LDH) Leakage Assay

Cell membrane damage was assessed by determining extracellular LDH release. Following EGCG exposure, culture supernatants were collected and incubated with the LDH reaction mixture provided in the assay kit. The absorbance was measured using a microplate reader, and LDH release was calculated as a percentage of total cellular LDH. Increased LDH release was interpreted as an indicator of compromised membrane integrity and enhanced cytotoxicity.

Statistical Analysis

Data are presented as **mean ± standard deviation (SD)** of three independent experiments. Graphical representation and descriptive statistical analysis were performed using **GraphPad Prism version 9.0** (GraphPad Software Inc., San Diego, CA, USA).

RESULTS:

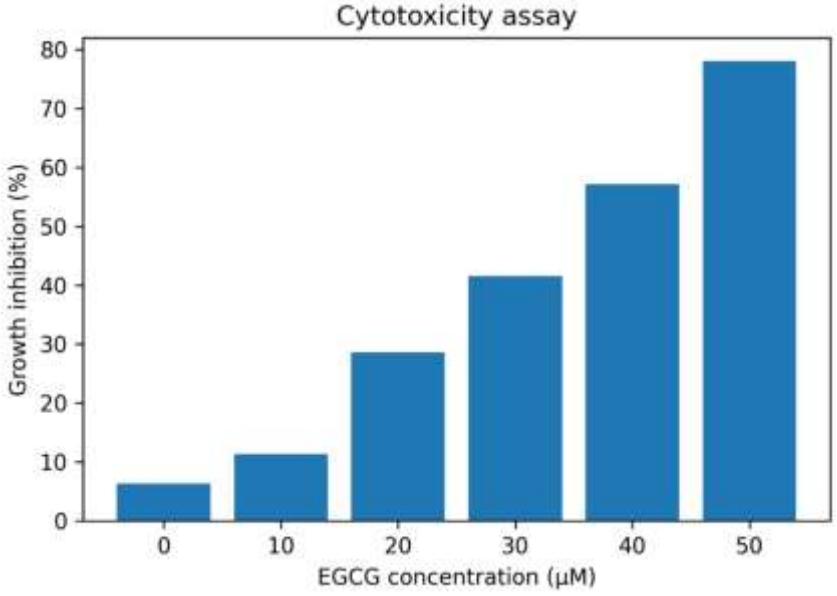
Effect of EGCG on MDA-MB-231 cell viability

The cytotoxic effect of EGCG on MDA-MB-231 cells was evaluated by measuring the percentage of growth inhibition following treatment with increasing concentrations of EGCG (0–50 µM). EGCG exhibited a concentration-dependent inhibitory effect on cell viability. Growth inhibition increased progressively from **6.37%** in the control group to **11.32%, 28.65%, 41.56%, 57.16%, and 78.06%** following treatment with **10, 20, 30, 40, and 50 µM** EGCG, respectively. The maximum inhibitory effect was observed at **50 µM**, demonstrating the potent cytotoxic activity of EGCG against MDA-MB-231 cells (Table 1; Figure 1).

Table 1. Effect of EGCG on cell viability of MDA-MB-231 cells

EGCG concentration (µM)	Growth inhibition (%)
Control (0)	6.37
10	11.32
20	28.65
30	41.56
40	57.16
50	78.06

Fig.**1: Cytotoxicity Assay on MDA-MB 231 Cells**



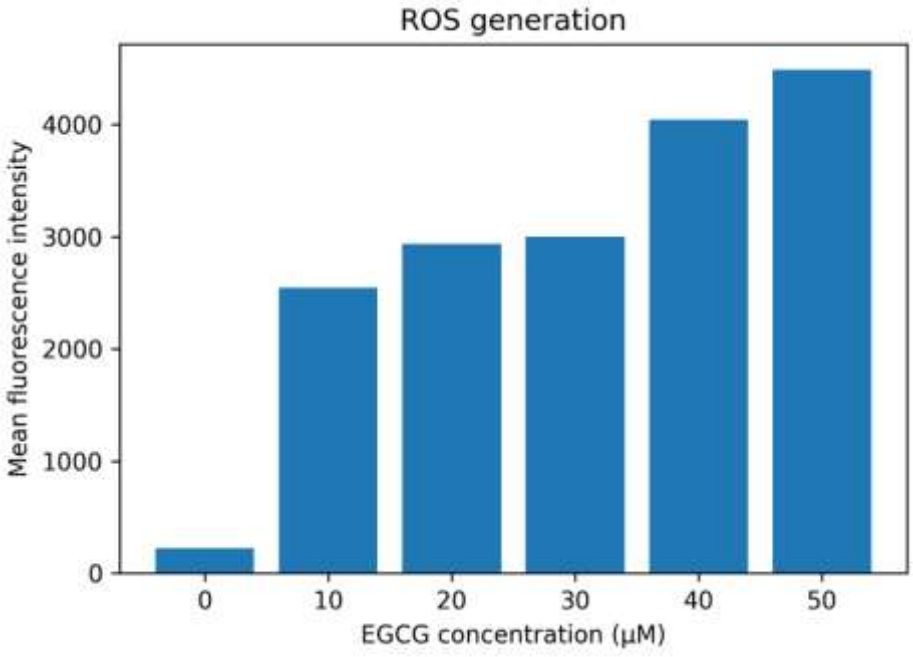
3.2 Effect of EGCG on intracellular ROS generation

Intracellular ROS production was quantified using the DCFH-DA assay. EGCG treatment produced a concentration-dependent increase in ROS generation. The mean fluorescence intensity increased from **220.5** in untreated cells to **2545, 2938, 3000, 4044.5, and 4491.5** following treatment with **10, 20, 30, 40, and 50 µM** EGCG, respectively, indicating progressive accumulation of intracellular ROS with increasing EGCG concentration (Table 2; Figure 2).

Table 2. Effect of EGCG on intracellular ROS generation in MDA-MB-231 cells

EGCG concentration (µM)	Mean fluorescence intensity (MFI)
Control (0)	220.5
10	2545
20	2938
30	3000
40	4044.5
50	4491.5

Fig.2: ROS Generation in MDA-MB-231 Cells (Quantitative DCFH-DA graph)



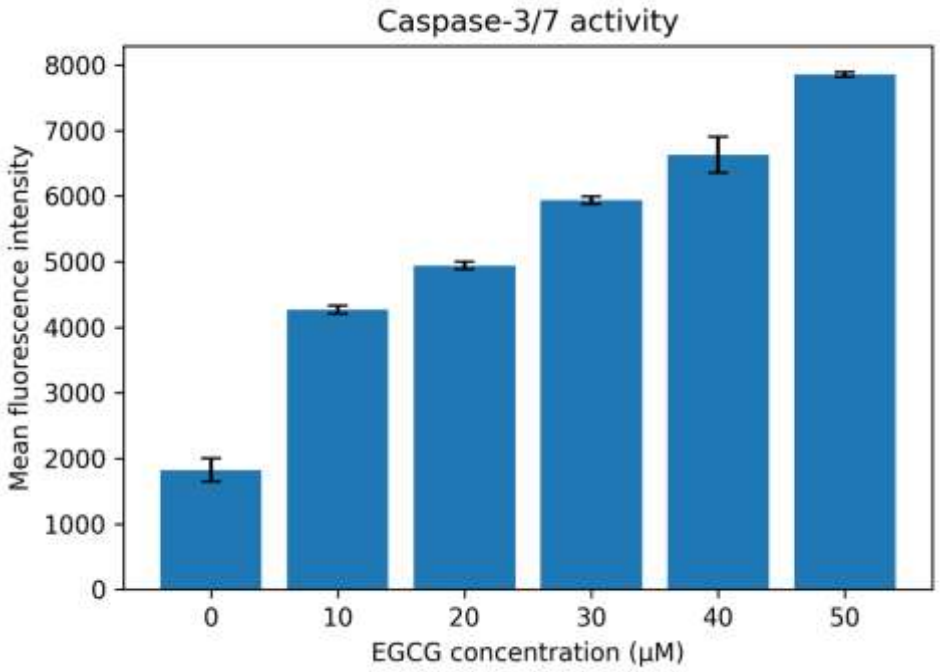
Effect of EGCG on caspase-3/7 activity

Caspase-3/7 activity increased progressively following EGCG treatment. Mean fluorescence intensity increased from **1822** in untreated cells to **4267, 4939, 5938, 6632, and 7860.5** after treatment with **10, 20, 30, 40, and 50 µM** EGCG, respectively. The highest caspase-3/7 activity was observed at **50 µM**, indicating concentration-dependent activation of the apoptotic pathway (Table 3; Figure 4).

Table 3. Effect of EGCG on caspase-3/7 activity

EGCG concentration (µM)	Mean fluorescence intensity (Mean ± SD)
Control (0)	1822 ± 173.21
10	4267 ± 62.35
20	4939 ± 57.74
30	5938 ± 56.58
40	6632 ± 277.13
50	7860.5 ± 36.37

Fig.3: Caspase-3/7 Activity in MDA-MB-231 Cells



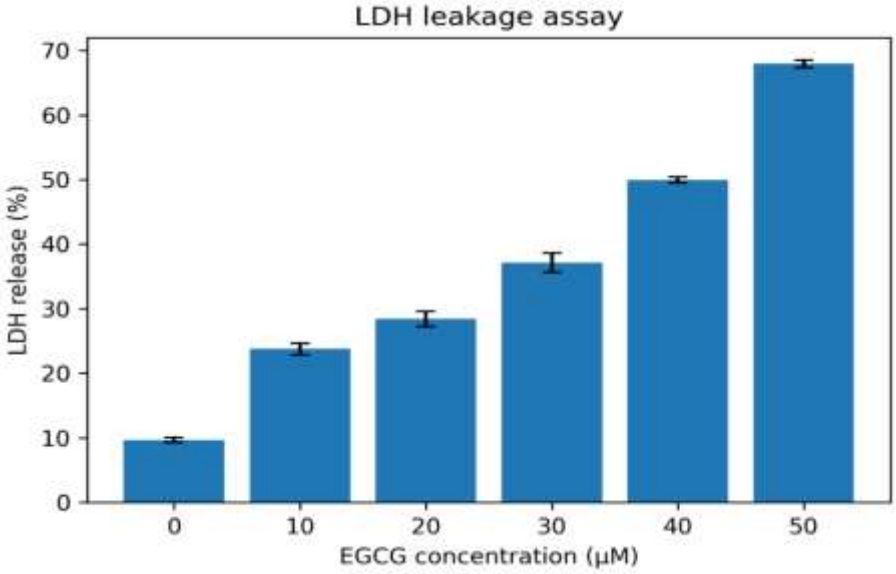
Effect of EGCG on LDH leakage

The effect of EGCG on membrane integrity was assessed by measuring LDH release. A concentration-dependent increase in extracellular LDH release was observed following EGCG treatment. LDH release increased from **9.65%** in untreated cells to **23.76%, 28.43%, 37.10%, 49.92%, and 67.93%** following treatment with **10, 20, 30, 40, and 50 µM** EGCG, respectively. The highest LDH release was recorded at **50 µM**, indicating increased membrane damage with increasing concentrations of EGCG (Table 4; Figure 4).

Table 4. Effect of EGCG on LDH leakage

EGCG concentration (µM)	**LDH release (%) (Mean ± SD)**
Control (0)	9.65 ± 0.40
10	23.76 ± 0.88
20	28.43 ± 1.18
30	37.10 ± 1.50
40	49.92 ± 0.48
50	67.93 ± 0.61

Fig.4: LDH Leakage Assay in MDA-MB-231 Cells



DISCUSSION

Triple-negative breast cancer (TNBC) remains one of the most challenging subtypes of breast cancer because of its aggressive clinical behaviour, high metastatic potential, and lack of hormone receptors and HER2 expression. These characteristics restrict the availability of targeted therapies, making the identification of novel therapeutic agents an important area of cancer research. In the present investigation, EGCG exerted a pronounced concentration-dependent inhibitory effect on MDA-MB-231 cells, accompanied by increased oxidative stress, activation of apoptosis, and disruption of cellular membrane integrity. Collectively, these observations indicate that EGCG suppresses TNBC cell survival primarily through oxidative stress-mediated apoptotic mechanisms.

The cytotoxicity assay demonstrated progressive inhibition of cell growth with increasing concentrations of EGCG, with maximum inhibition observed at 50 μM. These findings are consistent with previous reports demonstrating that EGCG inhibits proliferation of breast cancer cells by regulating multiple signalling pathways involved in cell survival, proliferation, angiogenesis, and metastasis. Suppression of PI3K/Akt, MAPK, NF-κB, STAT3, and Wnt/β-catenin signalling has been proposed as an important mechanism underlying its broad-spectrum anticancer activity. Similar observations have been reported by numerous experimental studies evaluating EGCG in breast cancer models. (20–22)

A notable finding of this study was the marked elevation of intracellular ROS following EGCG treatment. Although EGCG functions predominantly as an antioxidant under physiological conditions, it has been shown to exert selective pro-oxidant effects within malignant cells. Cancer cells generally maintain higher basal oxidative stress than normal cells, rendering them more susceptible to further ROS accumulation. Excessive ROS generation disturbs cellular redox homeostasis, resulting in oxidative injury to proteins, lipids, and nucleic acids, ultimately triggering mitochondrial dysfunction and apoptotic signalling. The concentration-dependent increase in ROS observed in this study is therefore consistent with the proposed mechanism of EGCG-induced oxidative stress reported previously. (23–26)

Enhanced ROS generation was accompanied by a substantial increase in caspase-3/7 activity, indicating activation of the intrinsic apoptotic pathway. Caspase-3 and caspase-7 function as executioner enzymes that mediate the irreversible phase of apoptosis by promoting chromatin condensation, DNA fragmentation, and apoptotic body formation. Increased oxidative stress promotes mitochondrial membrane depolarization and cytochrome *c* release, leading to sequential activation of initiator and executioner caspases. The observed dose-dependent increase in caspase-3/7 activity therefore supports apoptosis as a major mechanism responsible for the cytotoxic effects of EGCG. Comparable findings have been described in breast cancer as well as several other human malignancies treated with EGCG. (21,27–30)

Evaluation of membrane integrity using the LDH leakage assay further strengthened these observations. Progressive elevation of extracellular LDH release following EGCG exposure indicates increasing membrane damage accompanying apoptotic cell death. LDH is released when plasma membrane integrity is lost during the late stages of apoptosis or secondary necrosis and is widely accepted as an indicator of irreversible cellular injury. The concentration-dependent increase in LDH release observed in this study corresponds closely with the increases in ROS generation and caspase activation, suggesting that these events occur sequentially during EGCG-induced cell death. Similar findings have been documented in previous in vitro investigations evaluating EGCG-induced cytotoxicity. (31–33)

CONCLUSION:

The findings of this study demonstrate that EGCG possesses significant anticancer activity against MDA-MB-231 triple-negative breast cancer cells. Treatment with EGCG resulted in a marked reduction in cell viability together with increased intracellular ROS production, enhanced caspase-3/7 activity, and elevated LDH release, indicating induction of oxidative stress, activation of apoptosis, and loss of membrane integrity. These observations suggest that EGCG inhibits the growth of TNBC cells through coordinated modulation of multiple cellular pathways rather than a single mechanism.

Overall, the study highlights the potential of EGCG as a promising naturally derived therapeutic candidate for triple-negative breast cancer. However, further investigations using animal models and well-designed clinical studies are necessary to validate its efficacy, determine its safety profile, and establish its potential role in future cancer therapy.

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