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Research Article

Evaluation of Genistein's Cytotoxic and Apoptotic Effects on Human Foreskin Fibroblasts: A Molecular and Biochemical Analysis

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Abstract

Genistein, a naturally occurring isoflavone in soybeans, has gained attention for its phytoestrogenic and antioxidant properties. Its role in endocrine modulation, oxidative stress regulation, and apoptosis suggests therapeutic potential in cancer and metabolic disorders. Foreskin fibroblasts serve as a model for studying genistein's effects on cell proliferation, oxidative stress, and cytotoxicity. This study examines genistein's influence on gene expression related to cell growth and stress responses, offering insights into its therapeutic or adverse effects. Human foreskin samples from seven donors (6–12 years old) were used. Fibroblasts were isolated via collagenase and trypsin digestion and cultured in DMEM with 10% FBS. Cells were treated with genistein (20–100 µM), and viability was assessed using MTT assays. Antioxidant enzyme activities (SOD , GPx, CAT) were quantified spectrophotometrically. Gene expression of Bax, Bcl-2, IGF-1, and Casp3 was analyzed via real-time PCR. Data were evaluated using t-tests and ANOVA, with $p<0.05$ considered significant. Genistein induced dose- and time-dependent cytotoxicity, significantly reducing cell survival above 40 µM. IC50 values decreased over time, confirming enhanced cytotoxicity. Enzyme activity assays showed reduced CAT, SOD, and GPx levels. Gene analysis revealed upregulation of pro-apoptotic markers (BAX, Casp3) and downregulation of anti-apoptotic genes (Bcl-2, IGF-1), with a BAX/Bcl-2 ratio exceeding one, indicating apoptosis. These findings highlight genistein's potential as a therapeutic agent via oxidative stress modulation and apoptosis induction. This study confirms genistein's cytotoxic effects on fibroblast cells through oxidative stress and apoptosis. Further research is needed to clarify its molecular mechanisms and optimize therapeutic applications.

Keywords: Genistein; Cytotoxicity; Oxidative stress; Apoptosis.

1. Introduction

Genistein is a naturally occurring isoflavone primarily located in soybeans and various legumes, known for its wide range of biological effects and possible health advantages. As a phytoestrogen, it shares structural characteristics with estrogen, allowing it to engage with estrogen receptors within the body. This interaction may be crucial in regulating hormonal equilibrium, which has sparked considerable interest in its therapeutic applications [1]. Research has shown that genistein can alleviate menopausal symptoms and promote bone health, making it a focal point in studies aimed at understanding its role in hormone-related conditions [2].

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Genistein also has potent antioxidant properties, suggesting it may mitigate oxidative stress and inflammation within the body. Its dual action as an endocrine modulator and an antioxidant positions it as a valuable compound in health and disease management [3]. Investigations into its potential anticancer effects have garnered attention, with evidence indicating that genistein may inhibit the proliferation of specific cancer cell types while promoting apoptosis, particularly in breast and prostate cancers [4].

Overall, the multifaceted benefits of genistein, encompassing its antioxidant capabilities and potential anticancer effects, warrant further exploration [5]. It is currently being investigated for its potential advantages in cardiovascular health, metabolic regulation, and managing conditions such as osteoporosis and obesity. Genistein is available through dietary sources, particularly soy-based products like tofu, tempeh, and soy milk, as well as in supplement form [6]. Its potential benefits include addressing bone density and weight management [7].

Individuals can incorporate genistein into their diets by consuming soy products or considering dietary supplements [8]. Ongoing research aims to elucidate the mechanisms through which genistein exerts its effects, ultimately contributing to developing novel therapeutic approaches for managing oxidative stress, inflammation, and cancer [9]. The exploration of genistein's effects is a critical research frontier that could yield valuable insights into its role in health and disease management, highlighting the need for further investigation in this area [10-11].

Foreskin fibroblasts, originating from the connective tissues of newborn males' foreskin, provide a valuable model for examining the biological impacts of different substances on human cells [12]. They play a crucial role in the production of extracellular matrix components and the modulation of local growth factors, essential for effective tissue repair [13]. Studying these cells can lead to a better understanding of various pathological conditions and the development of targeted therapies, emphasizing their relevance in regenerative medicine [14].

Foreskin fibroblasts are primary cells isolated from human foreskin tissue. They are significant for cancer research due to their straightforward culture conditions and ability to replicate the fibroblastic elements found within tumors [15]. These cells can be manipulated to investigate various facets of cancer biology, including responses to oncogenic signals and the dynamics between tumor cells and their surrounding stroma .Thus, foreskin fibroblasts enhance our understanding of tumor biology and help identify novel therapeutic targets, making them crucial in advancing oncology research [16].

This research aims to explore the impact of genistein on the expression of various factors that play a role in cell proliferation, responses to oxidative stress, and cytotoxicity in human foreskin fibroblast cells. This study seeks to clarify how genistein affects the levels of critical growth factors, oxidative stress markers, and cytotoxicity proteins. Doing so will provide insights into the possible protective or harmful effects of genistein on fibroblast cells in different oxidative stress and growth scenarios. In particular, the investigation will focus on the modulation of gene expression related to cell growth and survival in the presence of genistein. The research will assess whether genistein enhances or inhibits the expression of these factors, contributing to a better understanding of its role in cellular processes. This could have significant implications for therapeutic strategies aimed at managing oxidative stress-related conditions. Ultimately, the findings from this study are expected to shed light on the dual nature of genistein's effects, potentially revealing its capacity to safeguard or compromise fibroblast cell integrity. By examining the interplay between genistein and the cellular mechanisms involved in growth and stress responses, the research aims to contribute valuable knowledge to cellular biology and therapeutic development.

2. Materials and Methods

Human foreskin samples were obtained from seven donors, aged between 6 and 12 years, who underwent standard circumcision procedures at the Akbar Children's Hospital in Mashhad, Iran. These tissue samples were collected with the approval from the institutional review board, ensuring compliance with the protocols set forth by the Clinical Research Institute at Mashhad University of Medical Sciences. Obtaining these samples was meticulously planned to align with ethical standards and institutional guidelines. Each donor's participation was voluntary, and all procedures were conducted to prioritize the safety and well-being of the children involved. The oversight by the institutional review board played a crucial role in maintaining the integrity of the research. This study received approval from the Club of Young and Elite Researchers under Grant number 4012587. The study received approval from the Research Ethics Committee at Mashhad University of Medical Sciences, under the identification number IR.IAU.MSHD.REC.1403.090.

2.1. Techniques for cell preparation and cultivation

The foreskin was meticulously excised to eliminate the underlying adipose tissue, which was then minced and rinsed with a phosphate-buffered saline (PBS) solution. The incubation time of two hours with a 0.25% collagenase solution was chosen to effectively digest the extracellular matrix and facilitate the isolation of individual cells from the foreskin tissue. Collagenase is an enzyme that breaks down collagen, a major component of the extracellular matrix, which is crucial for dissociating tissue into its cellular constituents. The two-hour incubation period allows sufficient time for collagenase to act on the tissue, ensuring efficient enzymatic digestion without causing excessive tissue degradation or compromising cell viability. The duration of collagenase treatment varies depending on the tissue type and the desired degree of dissociation. Studies have shown that a 1–2 hour incubation with collagenase can yield many viable cells, allowing the enzyme to effectively cleave collagen fibers while maintaining cell integrity [17]. This was followed by a brief 10-minute treatment with a 0.25% trypsin solution to facilitate cell dissociation. After the enzymatic treatments, the cells were separated from the supernatant using Dulbecco’s modified Eagle’s medium (DMEM) enriched with 10% fetal bovine serum (FBS). Cell quantification was conducted post-centrifugation using a hemocytometer, indicating a 1×10^5 cells per ml concentration in the DMEM medium containing 1% antibiotics and 10% FBS. The cells were maintained in a controlled environment at 37 degrees Celsius with 5% CO2 and were subsequently sub-cultured at a 1:3 ratio until they achieved 70-80% confluence. To detach the cells, a 0.5 ml aliquot of trypsin solution was added to the culture medium, and the cells were incubated for an additional 20 minutes under the same conditions. The resulting floating cells were then sub-cultured until the culture flask reached a density of 2×10^6 cells per cm^2. This study utilized fourth-generation fibroblasts to preserve cellular characteristics and ensure sufficient cell yield. After freezing and storage, the cells were cultured again after dilution to 2×10^5 cells per ml until they covered 70-80% of the surface area of a 25 cm^2 culture flask [18].

2.2. Preparation of genistein

Preparing genistein solutions at 20, 40, 60, 80, and 100 micromolar concentrations in Mashhad involves acquiring high-purity genistein powder from a trusted supplier. The amount of genistein needed is calculated based on its molecular weight, approximately 272.25 g/mol, alongside the desired final concentrations. An appropriate volume of dimethyl sulfoxide (DMSO) is chosen for the solution preparation for each concentration. To ensure accurate measurements, meticulous pipetting techniques are employed, and the solutions are thoroughly mixed to guarantee complete dissolution of the genistein. After preparation, the genistein solutions are aliquoted into smaller containers and stored in a cool, dark place to maintain their stability until needed for experimental purposes. This meticulous approach to handling and storage is essential for preserving the integrity of the solutions throughout the experimental period [19].

2.3. Assessment of cell viability using the MTT technique

The cells were incubated in a controlled environment at 37°C with 5% CO2 for 24, 48, or 72 hours. On the first, second, and third days of incubation, 20 ml of MTT was examined to assess cell viability. In the MTT assay, after removing the cell culture medium, the cells were washed with phosphate-buffered saline (PBS). They were then treated with a 0.5 mg/ml MTT solution, which is 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (Sigma), and incubated for 2 hours at 37°C. Following this incubation, the MTT solution was discarded, and the cells were lysed with 100 µl of dimethyl sulfoxide (DMSO) for subsequent analysis. The analysis was performed using a microplate reader (BioRad, California) set to a wavelength of 570 nm. The cell viability percentage was calculated using the formula: (mean absorbance of the treated sample/mean absorbance of the control sample) × 100, ensuring that the absorbance readings for each well were normalized against the blank absorbance value [20].

2.4. Assessment of the activity levels of SOD, GPx, and CAT

After 24 hours of treatment, the cells in the plate were detached from the well bottoms using trypsin. Following centrifugation, the resulting cell pellet was resuspended in PBS and subjected to another round of centrifugation. Subsequently, RIPA lysis buffer was introduced to the cell pellet, which was then aspirated to ensure complete dissolution in the lysis buffer and placed on ice for 30 minutes. The mixture was centrifuged at 10,000 rpm at -4 degrees Celsius for 15 minutes. The supernatant containing the enzyme extract was carefully collected and transferred to a new microtube.

The activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) were assessed in this study. Specifically, one unit of SOD was characterized as the quantity of enzyme that inhibited 50% of formazan production per minute. Oxanthine and xanthine oxidase were employed to generate superoxide anions. The interaction between these superoxide anions and tetrasodium chloride resulted in the formation of yellow formazan, which was quantified by measuring its absorbance at 450 nm. For the measurement of GPx activity, enzymatic reactions were conducted in test tubes containing reduced glutathione, glutathione reductase, and NADPH, with the addition of cumene hydroperoxide to initiate the reaction. The activity of GPx was determined by monitoring the absorbance at a wavelength of 340 nm. In the case of CAT, one unit was defined as the amount of enzyme that catalyzed the decomposition of 1 M of hydrogen peroxide (H_2O_2) per minute. The H_2O_2 decomposition rate was evaluated at 570 nm. The enzyme activities of SOD, GPx, and CAT were measured using assay kits from Jiancheng Bioengineering Institute and the results were expressed as units per milligram of protein (U/mg). All experimental procedures were conducted in triplicate to ensure the reliability and accuracy of the data obtained [21].

2.5. RNA extraction and cDNA synthesis

RNA extraction was carried out utilizing the Total RNA extraction kit provided by Yekta Tajhiz Azma, based in Tehran, Iran, adhering strictly to the manufacturer's specified protocols. The purity and concentration of the extracted RNA were assessed using a NanoDrop 1000 spectrophotometer from NanoDrop Technologies in Wilmington, DE, USA. Electrophoresis was performed on a denaturing 1.5% agarose gel to verify the integrity of the RNA. Acceptable A260/A280 ratios ranged from 1.8 to 2.1, with any samples exceeding 2.1 excluded from further analysis. The RNA samples underwent treatment with the DNase enzyme to eliminate potential DNA contamination, ensuring that RNase was absent throughout the procedure. After adjusting the RNA concentrations, cDNA synthesis was performed using the cDNA synthesis kit from TaKaRa, located in Otsu, Shiga, Japan. This synthesis step is vital for preparing the RNA for various applications in molecular biology. The synthesized cDNA was subsequently stored at -20°C until required for quantitative real-time PCR. The meticulous handling and processing of RNA and cDNA are critical for achieving reliable and reproducible outcomes in subsequent experimental applications [22].

2.6. Real-time PCR

The SYBR Green dye (KapaBiosystems, Inc., Wilmington, MA, USA) was utilized for real-time PCR on the Exicycler™96 Bioneer machine (Bioneer Corporation, Daejeon, Korea). Each PCR reaction was conducted in triplicate to ensure accuracy. The specific forward and reverse primers for amplifying the Bax, Bcl-2, IGF-1, and Caspase-3 genes are detailed in **Table 1**. The PCR reactions were prepared by combining the 2X KAPA SYBR FAST qPCR Master Mix (KapaBiosystems, Inc., Wilmington, MA, USA) with the respective primers and cDNA templates. The real-time PCR protocol included an initial denaturation step at 94°C for 10 minutes, followed by 40 amplification cycles, with each temperature maintained for 30 seconds. Subsequently, a melting curve analysis was conducted, transitioning from the annealing temperature to the denaturation temperature. Gene expression levels were quantified using the relative standard curve method and reported as fold changes in expression [23].

Table 1: Real-time PCR primers.

Gene name	Sequence	Length	Annealing temperature
GAPDH	Forward Primer: 5′ -TGAAGCAGGCATCTGAGGG-3′	19	67°C
	Reverse Primer: -5′ CGAAGGTGGAAGAGTGGGA-3′	19	67°C
Caspase-3	Forward Primer: 5′-TGGGTGCTATTGTGAGGCG-3′	19	69°C
	Reverse Primer: 5′ -GCACACCCACCGAAAACCA-3′	19	69°C
Bcl-2	Forward Primer: 5′-TGAACTGGGGGAGGATTGT-3′	19	66°C
	Reverse Primer: 5′ -AAATCAAACAGAGGCCGCA-3′	19	66°C
Bax	Forward Primer: 5′-CCCAGAGGCGGGGTTTCA-3′	18	63°C
	Reverse Primer: 5′ -GGAAAAAGACCTCTCGGG-3′	18	63°C
IGF-1	Forward Primer: 5′- CACCATGTCCTCCTCGCATCTC-3′	22	71°C
	Reverse Primer: 5′ - CCCTGTCTCCACACACGAACTG-3′	22	71°C

2.7. Statistical analysis

Statistical data are shown as mean ± SEM, with analyses conducted using unpaired t-tests or one-way and two-way ANOVA when suitable. A p-value of less than 0.05 was considered indicative of statistical significance.

3. Results and Discussion

3.1. MTT assay

The findings from this research highlight the impact of genistein on fibroblast cells, specifically regarding its cytotoxic effects and ability to inhibit cell growth. The survival rates of the treated fibroblast cells exhibited a notable contrast compared to the control group, indicating that genistein significantly influences cell viability. In the case of a 24-hour exposure to genistein, fibroblast cells demonstrated a clear dose-dependent growth inhibition. At a concentration of 20 µM, the cell survival rate was 91.5%. However, as the concentration of genistein increased, the survival rate declined, reaching only 19.5% at 100 µM. A marked difference in cell survival was evident at concentrations exceeding 40 µM compared to the control group. Similarly, during the 48-hour treatment period, fibroblast cell survival at 20 µM was 89.5%, which decreased to 16.5% at the highest concentration of 100 µM. A significant difference was again noted at concentrations above 40 µM. The trend continued in the 72-hour treatment, where survival rates fell from 81.4% at 20 µM to 15.6% at 100 µM. Statistical analysis confirmed that the reduction in survival rates at 20µM was significant compared to the control sample (**Figure 1**). The findings from these experiments indicate that the cytotoxic impact of genistein is enhanced as both the concentration and exposure time are increased (see **Figure 1**). This suggests that the substance's anticancer characteristics are influenced by the levels of concentration as well as the length of time it is applied. The relationship between genistein's effectiveness and its concentration is significant, with higher doses yielding a more pronounced cytotoxic response.

Additionally, the duration of exposure plays a crucial role in maximizing its anticancer potential. Overall, these results underscore the importance of concentration and time in determining the efficacy of genistein as a therapeutic agent against cancer. This information could be vital for optimizing treatment protocols that incorporate this compound.

The IC50 value for genistein was determined over various time intervals, specifically at 24, 48, and 72 hours. This metric indicates the concentration of genistein required to induce cell death in 50% of the population, while allowing the remaining 50% to survive. To derive the IC50 value, the percentage of cell survival is plotted against the concentration of genistein. By applying the equation of the resulting line and substituting the value of 50, one can ascertain the concentration at which 50% of the cells survive or perish. The calculated IC50 values for genistein are presented in **Table 2**. This analysis provides critical insights into the efficacy of genistein as a cytotoxic agent, highlighting its potential therapeutic applications. Understanding the concentration-dependent effects of genistein on cell viability is essential for further research and development in this area.

Table 2: Assessed the inhibitory concentration (IC50) of genistein

Duration of exposure (h)	IC 50 (µM)
24	48.79 ±1.5
48	45.89 ±1.2
72	42.74±1.05

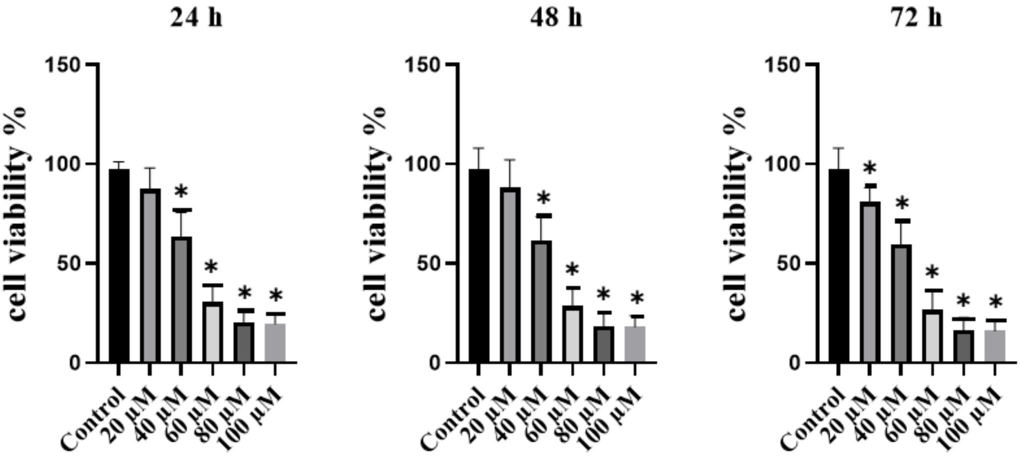

Figure 1. Assessment of fibroblast cell viability after exposure to different levels of genistein at 24, 48, and 72-hour intervals. * a notable distinction at the 5% significance level.

3.2. Evaluation of the functional levels of CAT, SOD, and GPx enzymes after a treatment duration of 24 hours

Figure 2 illustrates the activity levels of the enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT). Statistical analysis revealed a notable difference in CAT levels across various treatment groups compared to the control group. Specifically, all treatment groups significantly reduced CAT levels compared to the control. Furthermore, the statistical analysis highlighted a significant variation in SOD levels among the different treatment groups. In all groups administered genistein, there was a marked decrease in SOD levels when compared to the control group, indicating a consistent trend across the treatments. Lastly, the analysis demonstrated a significant difference in GPx levels among the treatment groups. In the group treated with genistein, GPx levels were significantly lower than those in the control group, reinforcing the observed trends in enzyme activity across the treatments.

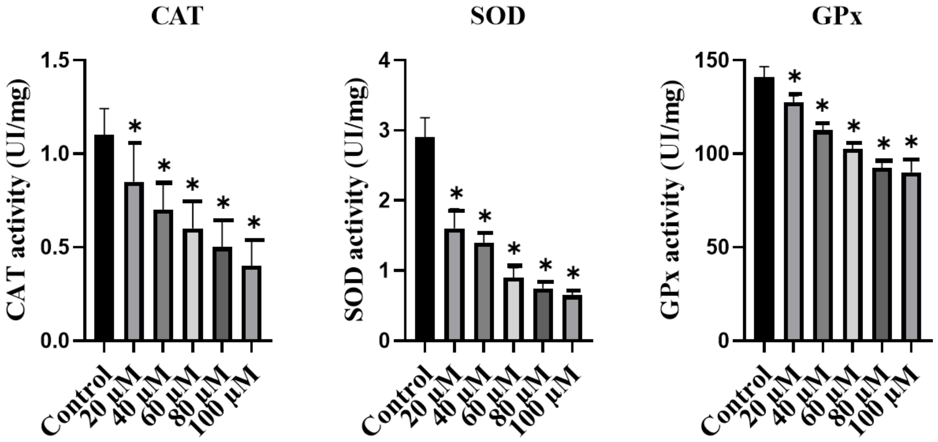

Figure 2. :Assessment of antioxidant enzyme activity for CAT, SOD, and GPx following a 24-hour treatment period. * a notable distinction at the 5% significance level.

3.3. Evaluation of the expression ratios of BAX, BCL-2, Casp3, and IGF-1 after a 24-hour treatment period

A real-time RT-PCR analysis was conducted with a minimum of three repetitions to assess the expression of apoptotic genes, specifically BAX and Casp3, alongside the anti-apoptotic genes Bcl-2 and IGF-1. GAPDH served as the reference gene to quantify the expression levels of each target gene. The results indicated a significant increase in the expression of BAX and Casp3 in the groups treated with genistein compared to the control group. Conversely, the anti-apoptotic genes Bcl-2 and IGF-1 expression levels exhibited a marked decrease. The ratio of BAX to Bcl-2 is typically used as a key indicator of a cell's propensity to undergo apoptosis, with a ratio exceeding one suggesting a heightened likelihood of apoptotic activity. This is particularly relevant given BAX's critical role in promoting apoptosis. Notably, the observed increase in the BAX/Bcl-2 ratio may be attributed to the reduced expression of Bcl-2, which normally functions to inhibit BAX. The diminished levels of Bcl-2 enhance the pro-apoptotic effects of BAX. The findings across all genistein-treated groups reveal that this ratio consistently exceeds one, thereby underscoring the pro-apoptotic influence of genistein (**Figure 3** and **Table 3**).

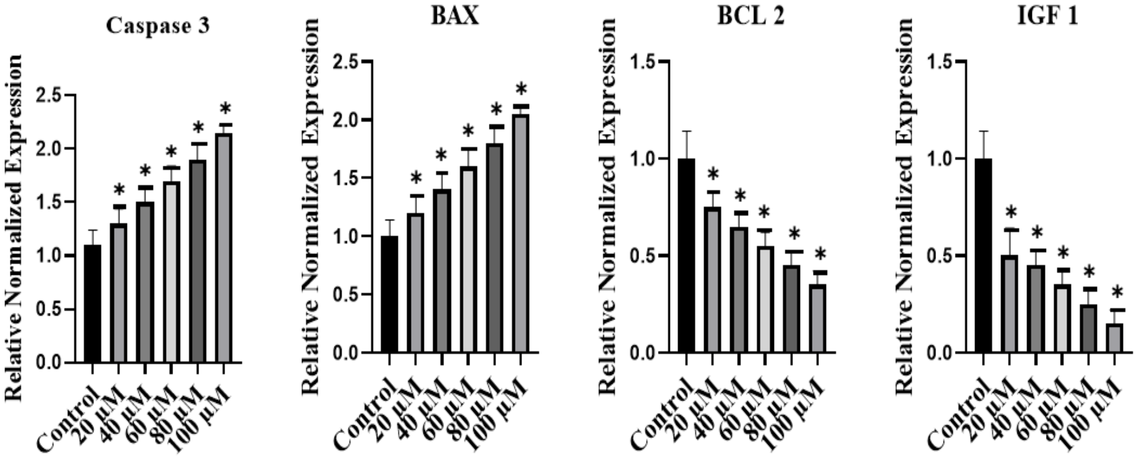

Figure 3. Assessing the expression levels of caspase 3, BCL-2, BAX, and IGF-1 genes. * a notable distinction at the 5% significance level.

Table 3: Influence of treatment concentration on the expression levels of caspase 3, BCL-2, BAX, and IGF1

Condition	Caspase 3	BAX	BCL 2	IGF 1
Control	1.0 ± 0.05	1.0 ± 0.05	1.0 ± 0.05	1.0 ± 0.05
20 µM	1.3 ± 0.12*, p=0.04	1.3 ± 0.11*, p=0.03	0.8 ± 0.10*, p=0.04	0.6 ± 0.08*, p=0.02
40 µM	1.5 ± 0.10*, p=0.01	1.5 ± 0.13*, p=0.02	0.7 ± 0.08*, p=0.01	0.5 ± 0.07*, p=0.008
60 µM	1.7 ± 0.09*, p=0.005	1.6 ± 0.09*, p=0.007	0.6 ± 0.06*, p=0.005	0.4 ± 0.05*, p=0.003
80 µM	1.8 ± 0.08*, p=0.001	1.8 ± 0.07*, p=0.002	0.5 ± 0.05*, p=0.001	0.3 ± 0.04*, p=0.001
100 µM	2.0 ± 0.06*, p=0.0005	2.0 ± 0.07*, p=0.0006	0.4 ± 0.03*, p=0.0003	0.2 ± 0.02*, p=0.0002

3.4. Discussion

The experimental findings indicate the cytotoxic effects of genistein, as evidenced by a significant decline in cell survival rates with higher concentrations. Notably, the survival rates of the treatment groups diverge markedly from those of the control group at concentrations above 40 µM, suggesting a critical threshold where genistein begins to demonstrate its cytotoxic properties. Previous studies have identified oxidative stress and apoptosis as possible mechanisms through which genistein induces cytotoxicity, ultimately leading to diminished cell viability [24]. The implications of these findings are far-reaching, particularly in the realms of oncology and regenerative medicine. Should genistein be confirmed to inhibit fibroblast proliferation while enhancing cytotoxicity, it may warrant further investigation as a potential anticancer agent. However, a significant challenge remains in the selective targeting cancerous fibroblasts, ensuring that normal cellular functions essential for maintaining tissue homeostasis are not adversely affected [25]. Furthermore, it is essential to explore genistein's influence on various signaling pathways associated with inflammation and cell survival. Its phytoestrogenic characteristics could have implications for hormone-sensitive tissues, necessitating careful evaluation in treatment scenarios, especially for patients diagnosed with hormone-sensitive cancers. This multifaceted approach will be crucial in understanding genistein's full therapeutic potential while mitigating any associated risks [26].

Genistein, a phytoestrogen in soy products, has attracted considerable attention due to its biological effects, particularly on fibroblast cells. The administration of genistein led to a notable decrease in the activity levels of the enzymes SOD, GPx, and CAT. Recent research has underscored its cytotoxic properties, notably through the alteration of antioxidant enzyme levels, including SOD, GPx, and CAT. The observed decrease in these antioxidant enzymes indicates that genistein may induce oxidative stress in fibroblast cells, which can have significant implications for cellular health and functionality [27]. When fibroblast cells are treated with genistein, there is a marked reduction in the activity of these critical antioxidant enzymes.

SOD plays a vital role in converting superoxide radicals into hydrogen peroxide and oxygen. At the same time, GPx is responsible for reducing hydrogen peroxide to water, and CAT further breaks down hydrogen peroxide. The downregulation of these enzymes suggests that the antioxidant defense system is overwhelmed, resulting in increased levels of reactive oxygen species (ROS). This disruption can push fibroblast cells towards apoptosis, as excessive ROS can damage cellular structures and interfere with normal signaling pathways [28]. The signaling mechanisms involved in these effects are intricate. A significant pathway affected by oxidative stress is the mitogen-activated protein kinase (MAPK) pathway, which encompasses p38 MAPK, ERK, and JNK. Activation of these pathways by ROS can lead to alterations in gene expression related to apoptosis and cell cycle control. In the context of genistein, the oxidative stress generated may trigger the activation of stress-response transcription factors such as Nrf2 and the tumor suppressor p53. While Nrf2 activation can promote the expression of antioxidant response elements, it may also lead to cell cycle arrest or programmed cell death in response to severe oxidative damage, thereby contributing to the overall toxicity associated with increased genistein levels [29].

The marked elevation in the levels of BAX and Casp3 in cells exposed to genistein underscores its involvement in facilitating apoptotic mechanisms. BAX, a vital pro-apoptotic protein within the Bcl-2 family, plays a key role in disrupting the mitochondrial membrane's integrity, which triggers the release of cytochrome c and initiates downstream apoptotic pathways. The increased expression of Casp3, an executioner caspase, further reinforces that genistein treatment advances the apoptotic process [30]. In contrast, the significant reduction in the expression of anti-apoptotic genes such as Bcl-2 and IGF-1 aligns with this pro-apoptotic effect. Bcl-2 typically functions to counteract pro-apoptotic signals, including those from BAX, thereby serving as a protective barrier against apoptosis. A decrease in Bcl-2 levels diminishes its inhibitory influence on BAX, promoting a more robust apoptotic signaling cascade [31]. The ratio of BAX to Bcl-2 is a critical metric for assessing a cell's vulnerability to apoptosis. A ratio exceeding one indicates that pro-apoptotic signals are predominant over anti-apoptotic factors, suggesting a heightened likelihood of programmed cell death. The findings that this ratio consistently surpasses one in all groups treated with genistein strongly suggest that genistein effectively shifts the balance in favor of apoptosis [32].

The transition in cancer treatment strategies is particularly significant, as many therapies focus on inducing apoptosis in cancerous cells while safeguarding normal tissues from adverse effects. Genistein may play a crucial role in this context by enhancing the BAX/Bcl-2 ratio, which could facilitate the death of malignant cells. This mechanism highlights its potential contribution to anticancer effects. However, it is essential to consider the consequences of promoting apoptosis in non-cancerous cells, such as fibroblasts, which are vital for tissue repair and maintaining homeostasis [33]. When fibroblast cells are exposed to genistein, the expression of pro-apoptotic genes is often increased, notably BAX and caspase 3. The upregulation of caspase 3, a pivotal component in the apoptotic process, indicates that genistein activates the intrinsic apoptotic pathway by encouraging the release of pro-apoptotic factors from the mitochondria. This activation leads to significant cellular changes, including chromatin condensation and DNA fragmentation, which are characteristic of apoptosis and demonstrate genistein's effectiveness in initiating these apoptotic mechanisms [34]. In addition to its pro-apoptotic effects, genistein also significantly influences anti-apoptotic factors. Research has shown that genistein can substantially reduce the expression of Bcl-2 and IGF-1, essential for cell survival. Bcl-2 functions as a primary antagonist to BAX, maintaining mitochondrial integrity and preventing apoptosis; thus, lower levels of Bcl-2 increase the likelihood of cells responding to apoptotic signals triggered by genistein. Similarly, reducing IGF-1 levels weakens cell survival signals, protecting cells from death. This dual action of decreasing anti-apoptotic signaling while enhancing pro-apoptotic activity effectively shifts the balance towards apoptosis, rendering cells more susceptible to genistein-induced cell death [35].

The interplay between caspase 3, BAX, and IGF-1 in genistein treatment highlights a sophisticated network of signaling pathways that regulate cellular responses to stress. Genistein functions as an inducer of apoptosis through the activation of caspases and as a regulator of essential survival pathways, particularly those influenced by IGF-1. This dual functionality significantly promotes a pro-apoptotic environment within cells, with an increased BAX/Bcl-2 ratio as a crucial marker of heightened apoptotic activity [36]. Gaining insight into these interactions is essential for leveraging the therapeutic capabilities of genistein, especially in formulating targeted cancer treatments that can utilize apoptotic mechanisms to eradicate malignant cells while safeguarding normal tissue effectively. As ongoing research delves deeper into these pathways, it will enhance the understanding and optimization of genistein's use in clinical applications, potentially leading to more effective cancer therapies [37].

4. Conclusion

In summary, the current investigation reveals that genistein has notable cytotoxic effects on fibroblast cells, which are influenced by both dosage and duration of exposure, as demonstrated by the MTT assay findings. The observed decrease in cell viability correlating with higher concentrations of genistein and extended treatment periods indicates its promising role as an anticancer agent. Furthermore, assessments of enzymatic activity show a significant decline in levels of CAT, SOD, and GPx, pointing to a mechanism of oxidative stress that may be responsible for the cytotoxic effects induced by genistein. The gene expression analysis further corroborates these results, showing an increase in pro-apoptotic genes such as BAX and Casp3, alongside a decrease in anti-apoptotic genes like Bcl-2 and IGF-1. This shift leads to a higher BAX/Bcl-2 ratio, which is conducive to promoting apoptosis. These observations collectively underscore the complex role of genistein in enhancing oxidative stress and activating apoptotic pathways, thereby highlighting its potential as a therapeutic agent in cancer treatment. Future studies need to delve deeper into the specific molecular mechanisms that drive genistein-induced cytotoxicity. Such research will be crucial in refining its clinical applications and maximizing its effectiveness as a cancer treatment option. Understanding these underlying processes will enhance our knowledge of genistein's action and pave the way for its integration into therapeutic strategies against cancer.

Ethical approval

The research aligned with the ethical standards outlined in the Helsinki Declaration, ensuring that all procedures adhered to the relevant guidelines and regulations as approved by the Islamic Azad University of Mashhad Medical Sciences Branch. Ethical approval and the necessary license were secured from the Research Deputy Ethics Committee of the institution, identified by the number 4012587 and the code IR.IAU.MSHD.REC.1403.090.

Artificial intelligence utilization for article writing

Artificial intelligence (AI) has not been used for writing this article.

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Conflict of interest statement

The authors declare that they have no competing interests.

Data availability

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

Author contributions

MP was instrumental in the collection of samples and the execution of tests, in addition to aiding in the development of the methodology and the preliminary draft of the manuscript. **ST** contributed by assisting in sample collection and manuscript writing. **JKH** and **FM** played significant roles in the study's conception and experimental design. **MP** took charge of data curation, validation, statistical analysis, and the preparation of the manuscript, whereas **JKH** concentrated on reviewing and refining the written material.

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Using artificial intelligence chatbots

There was no use of artificial intelligence in the making of this article.

Reference

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