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RESEARCH

Impact of genistein on androgen-independent PC3 prostate cancer cells

Genisteinin androjen-bağımsız PC3 prostat kanseri hücreleri üzerindeki etkisi

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Abstract

Purpose: This study evaluates genistein's effects on cell survival, migration, apoptosis, reactive oxygen species (ROS) generation, and Manganese Superoxide Dismutase (MnSOD) expression in androgen-independent PC3 prostate cancer cells, providing insight into its potential as an adjuvant therapy for castration-resistant prostate cancer (CRPC).

Materials and Methods: Cells were treated with vehicle only and 0.5, 2.5, 5, 10, and 50 μM genistein concentrations for 24 and 48 hours. Cell proliferation assay, wound healing assay, ROS measurement, apoptosis detection, and MnSOD protein expression analysis were performed.

Results: The findings indicate a biphasic effect of genistein on PC3 cell survival. Lower to physiologically relevant concentrations (0.5–10 μM) exhibit a modest stimulatory effect, whereas a higher, pharmacologically achievable concentration (50 μM) leads to a time-dependent decline in survival and a significant restriction on migration. In vehicle-treated cells, 77% remained viable, with low early (3.65%) and late apoptosis (16.35%). Lower genistein concentrations (0.5–10 μM) caused a slight increase in apoptosis and a modest decline in viability. However, at 50 μM, only 18.7% of cells remained viable, while 74.25% underwent late apoptosis or cell death.

Conclusion: These findings demonstrate that genistein, particularly at higher concentrations, inhibits androgen-independent PC3 cell growth through apoptosis induction, MnSOD regulation, and elevated oxidative stress.

Keywords: Androgen-independent prostate cancer cells, genistein, cell migration, ROS production, cell survival, apoptosis.

Öz

Amaç: Bu çalışma, genisteinin andojen-bağımsız prostat kanser hücrelerinde hücre sağkalımını ve migrasyonunu, apoptozu, reaktif oksijen türleri (ROS) oluşumunu, antioksidan protein Manganez Süperoksit Dismutaz (MnSOD) ekspresyonunu nasıl etkilediğini göstermeyi ve özellikle kastrasyona dirençli prostat kanser adjuvan tedavisi olası kullanımına ışık tutmayı amaçlamaktadır.

Gereç ve Yöntem: Hücreler, 24 ve 48. saatlerde genistein bulunmayan ve 0.5, 2.5, 5, 10 ve 50 μM konsantrasyonlarda genistein bulunan ortamlarda büyütüldü. Sonuçları değerlendirmek için hücre proliferasyon analizi, hücre migrasyon analizi, reaktif oksijen türleri üretim ölçümü, apoptoz tespiti ve MnSOD protein ekspresyon analizi yapıldı.

Bulgular: Bulgularımız, genisteinin PC3 hücreleri üzerinde bifazik etkileri olduğunu göstermiştir. Özellikle daha düşük-fizyolojik konsantrasyonlarda (0,5-10 μM) ılımlı bir uyarıcı özellik gösterdiği, daha yüksek, farmakolojik (50 μM) konsantrasyonda hücre sağkalımında düşüş ve hücre göçünde önemli bir kısıtlamaya sebep olduğu saptanmıştır.

Sonuç: Analiz sonuçları genisteinin, özellikle yüksek konsantrasyonda, androjen-bağımsız PC3 prostat kanseri hücrelerinin büyümesini, apoptoz indüksiyonu, MnSOD regülasyonu ve yüksek oksidatif stres gibi süreçler yoluyla inhibe ettiğini göstermektedir.

Anahtar kelimeler: Androjen-bağımsız prostat kanseri hücreleri, genistein, hücre göçü, ROS üretimi, hücre sağkalımı, apoptoz.

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INTRODUCTION

Prostate cancer, one of the most prevalent malignancies and a leading cause of cancer-related deaths in men is a significant global health concern[1]. Although localized prostate cancer can be treated initially by androgen deprivation therapy (ADT), a substantial percentage of cases progress into castration-resistant prostate cancer (CRPC), which is characterized by aggressive behavior and resistance to conventional therapies[2]. This phenomenon emphasizes the critical need for novel treatment strategies focusing on the unique molecular causes of CRPC development.

Genistein, a naturally occurring isoflavone found in soy, has attracted much interest due to its possible therapeutic and chemopreventive effects against several malignancies, including prostate cancer[3]. According to epidemiological data, diets high in soy isoflavones may lower the incidence of prostate cancer, especially in Asian populations where soy consumption is traditionally high[4]. The various anti-cancer mechanisms of genistein include regulating several biological functions, such as angiogenesis, oxidative stress, cell migration, proliferation, and apoptosis[5]. Genistein's capacity to cause cancerous cells to go through apoptosis is crucial to its anti-cancer action. Research has demonstrated that genistein can cause prostate cancer cells to undergo apoptosis via various mechanisms, such as inhibiting focal adhesion kinase (FAK) activity, a crucial regulator of cell survival signaling[6]. Furthermore, a key factor in genistein's anti-cancer properties is its impact on producing reactive oxygen species (ROS).

At physiological levels, ROS serve as signaling molecules, but when they are produced in excess, they can cause oxidative stress and apoptosis. It has been shown that genistein alters ROS levels, which causes cancer cells to undergo oxidative stress-induced apoptosis [7]. It is crucial to understand how genistein affects the delicate balance between antioxidant defense and the production of ROS generation. Manganese superoxide dismutase (MnSOD), a chief ROS-scavenging mitochondrial antioxidant enzyme, has a crucial role in regulating intracellular ROS levels in the cell. Lung cancer is one of the cancer types where genistein has been found to decrease stem-like features via altering MnSOD expression [8]. Determining genistein's possible therapeutic role in prostate cancer requires an understanding of how it affects MnSOD regulation.

In addition to oxidative stress and apoptosis, genistein has been shown to have inhibitory effects on cell migration and proliferation, two processes essential for cancer advancement. A thorough analysis emphasized the diverse chemotherapeutic potential of genistein, which includes angiogenesis suppression, apoptosis induction, and cell cycle modification [9]. Given that CRPC is an aggressive and metastatic cancer, these combined effects make genistein an attractive possibility for addressing this disease. Genistein has the potential to be a treatment drug for CRPC because of these complex modes of action. To maximize its therapeutic potential and reduce any potential off-target effects, a comprehensive understanding of its mechanisms of action is necessary due to the concentration-dependent nature of its behavior.

This study aims to investigate the effects of genistein on apoptosis, ROS generation, cell migration, MnSOD expression, and cell proliferation in androgen-independent prostate cancer cells, thereby providing a foundation for further translational research. Our hypothesis is that genistein exerts a dose-dependent biphasic effect on androgen-independent prostate cancer cells, where its ROS-generating and anti-migratory properties may enhance its potential as an adjuvant therapy for CRPC by increasing cancer cell sensitivity to treatment. This study offers novel insights into literature regarding the physiological and pharmacological dose-dependent biphasic effects of genistein on PC3 cells. Specifically, it demonstrates that genistein exhibits potential anti-metastatic effects by modulating oxidative stress through MnSOD regulation, inducing ROS-mediated cytotoxicity and apoptosis. These findings suggest that genistein may serve as a potential adjuvant therapy to enhance the efficacy of chemotherapy in prostate cancer treatment.

MATERIALS AND METHODS

This research was conducted at Bezmialem Vakıf University's Experimental Application and Research Center, which facilitates comprehensive studies across a variety of scientific disciplines. File reliability is ensured through high-security protocols in data management. Research data are accessible only to authorized personnel, and analysis processes are conducted in accordance with national and international scientific ethical standards by trained researchers. Each stage is documented transparently.

Cell culture

Human PC3 prostate cancer cells were cultivated in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) and 1% Penicillin/Streptomycin in a humidified incubator at 37°C containing 5% CO_2. Medium, FBS and Penicillin/Streptomycin were purchased from Gibco (Carlsbad, CA, USA). Dr. Bosland from the University of Illinois at Chicago gifted the PC3 cell lines. Exponential growing cells (60% to 70% confluence) were exposed to 0.5, 1μM, 2.5μM, 5μM, 10μM, 25μM, and 50 μM concentrations of genistein (Sigma, St Louis, MO, USA) dissolved in dimethylsulfoxide and the vehicle served as the control.

Cell proliferation assay

To evaluate the effect of genistein treatment on the growth of PC3 cells, the WST-1 calorimetric cell proliferation assay kit (ROCHE) was used. The cells were seeded at a density of 1×10^4 cells per well and incubated with 100 μL of culture medium overnight in a 96-well plate. Subsequently, 0.5, 1, 2.5, 5, 10, 25, and 50 μM genistein concentrations were added. After 24 and 48 hours, each well was incubated with 10 μL WST-1 reagent for 4 hours at 37°C. Absorbance was measured at 450 nm OD using a microplate reader (Thermo Fisher Scientific, England, Multiscan GO). The assay was run in triplicates.

Cell migration assay

PC3 cell migration after genistein treatment was performed by wound healing assay, which is a method to measure the status of migration and cell proliferation by disrupting confluent cell monolayers[10]. Wound closure of PC3 cells was observed after 0, 24 and 48 hours. Cells were seeded at a density of 2×10^5 cells/well in 6-well plates. Cells were washed with PBS the next day, scratched with a 200 μL yellow tip, and treated with genistein at concentrations of 0.5, 2.5, 5, 10, and 50 μM, which were selected based on the results of the cell proliferation assay. Scratch closure was calculated using the nearest scratch edge distance in each field [11]. Briefly, a phase-contrast inverted light microscope at 20X magnification (Zeiss Primovert) was used to image each well at 0, 24, and 48 hours. Subsequently, the 24 and 48 h images have been enhanced with the two parallel solid black lines drawn at the edges of the wound image at 0 h. This was done using the Image J program. Finally, the Image J program (NIH, Bethesda, USA) was used to identify at least three separate areas within the wound and ten measurements were taken per area.

Reactive oxygen species (ROS) generation detection

The fluorogenic probe 6-carboxy-2',7'-dichlorodihydrofluorescein diacetate (carboxy-H_2DCFDA; Invitrogen, Carlsbad, CA) was used to measure intracellular ROS levels. A stock solution of 10 mg/mL carboxy-H_2DCFDA in DMSO was prepared, aliquoted in 60-μL portions, and stored at -20°C. Each aliquot was thawed only once before use to maintain reagent stability. PC3 cells were incubated with 10 μM carboxy-HDCFDA in a culture medium at 37°C in the dark for 30 minutes. After incubation, cells were rinsed with 1X PBS to remove excess probe and then treated with 0.5μM, 1μM, 2μM, 5μM, 10μM, and 50μM concentrations of genistein. Measurements were made at 5-minute intervals for 1 hour at 37°C using a microplate reader (excitation: 492 nm, emission: 527 nm) (Thermo Fisher Scientific, England). Based on the data taken at the 1-hour time point, the fluorescence intensity of oxidized carboxy-H_2DCF—which has a direct correlation with intracellular ROS levels—was measured, and the results were evaluated.

Apoptosis detection

The Muse Annexin V & Dead Cell Assay Kit (Merck Millipore, Germany) was used to assess apoptosis using the Muse Cell Analyzer (Merck Millipore, Germany). This assay uses a premixed Annexin V combined with a dead cell marker, 7-amino actinomycin (7-AAD). PC3 cells were treated with vehicle alone and with genistein at concentrations of 0.5, 2.5, 5, 10, and 50 μM for 48 hours. Cells were combined with assay reagents and incubated for 20 minutes in the dark at room temperature after pelleting by centrifugation. Data were displayed as percentage of live, early apoptotic, late apoptotic, or dead cells as described previously [12].

Western blotting

Protein expression analysis was assessed by Western blot method. Cells were seeded at a density of 1×10^6 cells and then treated for 48 h with vehicle only, and as well as 0.5, 2.5, 5, 10 and 50 μM genistein concentrations. 1X Cell lysis buffer (Cell Signaling

Technology, USA) containing diluted 1 mM PMSF (Sigma, Germany) in distilled water was used for lysing of treated and vehicle cells after harvesting. Protein concentrations were measured by BCA assay kit in the MultiSkan GO Microplate Spectrophotometer (Thermo Fisher Scientific, England). Sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE) were used to separate proteins (20μg) before being transferred to a PVDF membrane. Afterwards, the blots were incubated with β-actin (Cell Signaling Technology, USA) and the primary antibody against Manganese Superoxide Dismutase (MnSOD) (Abcam, Cambridge, US). After secondary antibody incubations, to visualize the specific binding, blots were treated with chemiluminescence solution. Protein bands were measured densitometrically by Image J (NIH, Bethesda, USA). The data were normalized relative to β-actin expression.

Statistical analysis

The findings of the analyses are displayed as a mean ± standard error of the mean by using the software system GraphPad Prism 7® (La Jolla, CA, USA). The group differences were evaluated using Student's t-test, which compares the means of two groups, and One-way Analysis of Variance (ANOVA), which assesses whether there is a significant difference between the means of the groups being compared. A *p-value* $\leq$ *0.05*, $p <$ *0.01*, and $p \leq$ *0.0001* were considered statistically significant. Each significance level is represented by an asterisk under the experiment results legends.

RESULTS

Biphasic effects of genistein on PC3 cell survival

WST-1 assay was used to evaluate proliferation and cytotoxicity of genistein in PC3 cells, (Figure 1). The impact of genistein on PC3 prostate cancer cell growth was evaluated at 24 and 48 hours across concentrations between 0.5–50 μM. Genistein exhibited a slight increase in cell growth at lower physiological concentrations (0.5–5 μM) compared to the vehicle control. The significant viability was reported at 10 μM, indicating a possible proliferative impact on this dose. However, the maximum reduction in viability was observed in a dose-dependent manner, suggesting cytotoxic effects at higher pharmacological concentrations (25 and 50 μM). Additionally, genistein treatment at 48 hours showed a notable reduction in proliferation compared to the 24-hour treatment group, indicating a time-dependent response. Significant variations from the control were revealed by statistical analysis, especially at 10 μM ($p <$ *0.01*) and at 25 and 50 μM ($p <$ *0.001*). These results suggest that genistein has biphasic effects on PC3 cells, stimulating viability at low doses and exhibiting time-dependent cytotoxicity at higher concentrations.

Suppression of PC3 cell migration by genistein in a dose and time-dependent fashion

Evaluating the effect of genistein on PC3 prostate cancer cell migration at different concentrations (0.5–50 μM) over 0-, 24-, and 48-hour time intervals was performed by wound healing assay (Figure 2). Representative images indicate that, in the vehicle control group, the wound area progressively closed over time, showing active cell migration (Figure 2A). Conversely, treatment with increasing concentrations of genistein led to a dose-dependent reduction in wound closure. Notably, at higher concentrations (10 and 50 μM), minimal migration was observed, with a significant inhibition of wound healing at 48 hours. Quantitative analysis of relative scratch width (Figure 2B) further confirmed the inhibitory effects of genistein. Compared to vehicle control, PC3 cells treated with 2.5 μM genistein exhibited a modest reduction in migration. Treatment with 10 and 50 μM resulted in a statistically significant ($p <$ *0.01 and* $p <$ *0.001*, respectively) increase in relative scratch width, exhibiting impaired migratory capacity. These results demonstrate that genistein suppresses PC3 cell migration in a dose- and time-dependent manner, underscoring its potential to impede prostate cancer cell motility.

Cell death induced by genistein in PC3 cells

To assess the apoptotic patterns of PC3 cells, Annexin V/7-AAD flow cytometry analysis was performed following treatment with increasing concentrations of genistein (0.5–50 μM) (Figure 3). A representative scatter plot in the quadrants illustrates the distribution of live, early apoptotic (Annexin V positive, 7-AAD negative), late apoptotic/dead (Annexin V positive, 7-AAD positive), and dead cells across treatment conditions respectively. In the vehicle-treated group, the majority of PC3 cells

remained viable (77%), with a low percentage undergoing early (3.65%) or late apoptosis (16.35%). Lower concentrations of genistein treatment (0.5–10 μM) indicated a slight increase in apoptotic cell populations, with a modest reduction in viable cells. However, a dramatic shift was observed at the 50 μM highest concentration, with only 18.7% of the cell population remaining viable, while 74.25% of the cells underwent late apoptosis or cell death. The results demonstrate that genistein causes apoptosis in PC3 cells in a dose-dependent fashion, with physiological- low to moderate concentrations (2.5-10 μM) causing gradual apoptotic changes and a pharmacological high dose (50 μM) leading to extensive cell death exhibiting its potential as an anti-cancer agent targeting prostate cancer cell viability.

Increased ROS generation by genistein-treated PC3 cells

To evaluate the impact of genistein on intracellular ROS generation in PC3 cells, the levels of ROS following treatment with varying concentrations of genistein (0.5-50 μM) were assessed (Figure 4). The results show that genistein has a concentration-dependent influence on ROS accumulation in PC3 cells. Lower genistein concentrations (0.5-5 μM) treatment resulted in levels of ROS relative to the vehicle control, with a slight, non-significant increase observed at 2.5 μM. However, higher concentrations (10 and 50 μM) ($p < 0.01$) led to a moderate to significant increase in ROS generation. This data demonstrates that genistein exhibits a biphasic effect on ROS generation in PC3 cells. While lower concentrations appear to have minimal impact, higher concentrations induce a significant increase in ROS production, potentially contributing to the observed anti-cancer effects of genistein in these cells.

Variations in MnSOD protein expression following genistein treatment

The results show a dose-dependent trend in the expression of MnSOD in genistein-treated PC3 cells (Figure 5). At baseline, with the vehicle treatment, the MNSOD/β-actin ratio was 0.448. At increasing genistein concentrations the ratio went up, reaching 0.539 at 2.5 μM ($p < 0.001$). Higher concentrations, however, showed some variation: particularly MnSOD expression peaked at 0.631 at 50 μM ($p < 0.001$) and was 0.480 at 5 μM, and 0.505 at 10 μM. This pattern indicates a potential stimulatory effect on MnSOD expression at higher genistein concentrations in PC3 cells. This data suggests that genistein may influence MnSOD levels in a dose-dependent manner, potentially affecting oxidative stress control and associated metabolic processes.

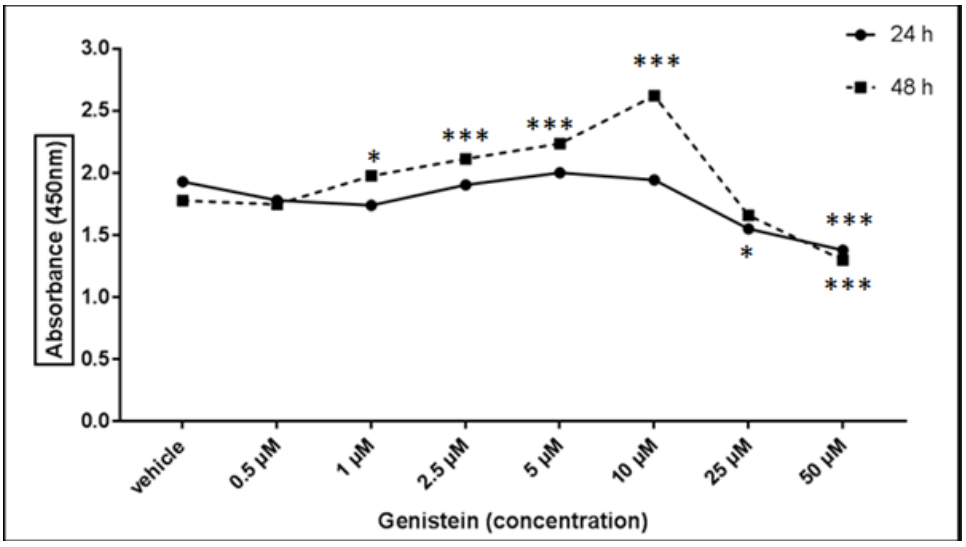

Figure 1. Cell proliferation was determined by the WST-1 assay. Triplicate wells containing 1 × 10^4 cells/well of PC3 were exposed 0.5,1, 2.5, 5, 10, 25 and 50 μM concentrations of genistein. Growth curves were obtained at 48 hr. Error bars represent the SEM values. $p < 0.05$ * $p < 0.001$* * and $p < 0.001$* * * represents statistically significant.

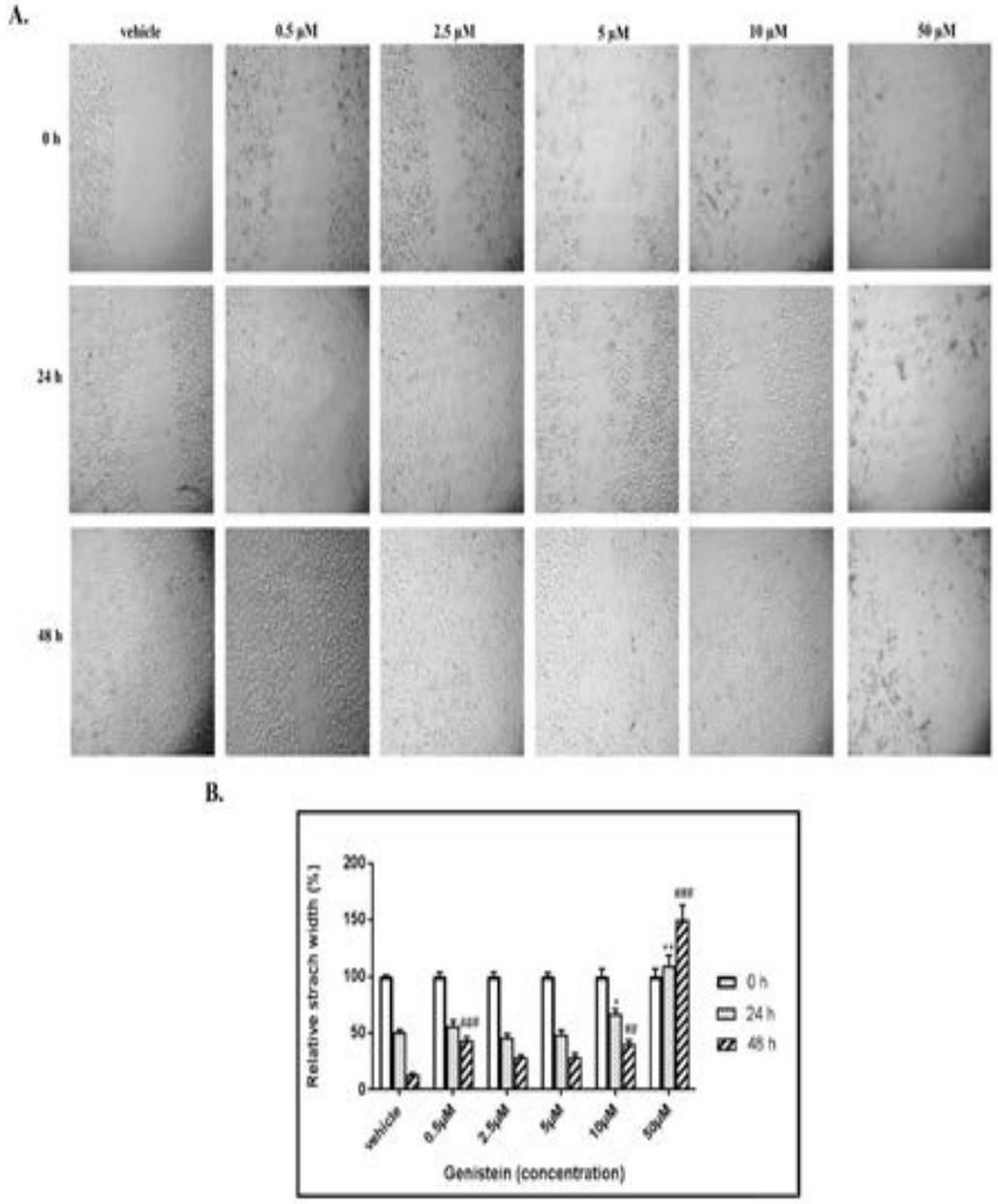

Figure 2. Cell migration was assessed by wound healing assay. A) PC3 cells were treated with 0.5, 2.5, 5, 10, and 50 µM concentrations of genistein at 0, 24 and 48 hours. Images were taken at 20x magnification. B) Quantification of migrating cells. Each bar represents quantification of mean distance migration ± *SEM*. Black lines on the digital images designate the borders of wound at the beginning of migration assay. $p < 0.05$, $p < 0.01$, $p < 0.001$*** represents statistically significant.**

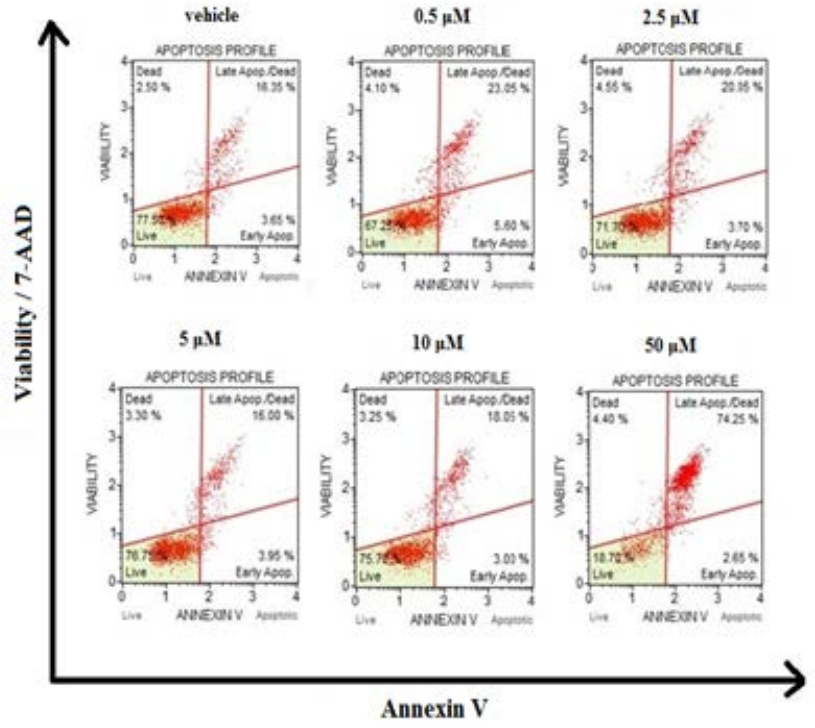

Figure 3. Analysis of apoptosis and cell death were evaluated in genistein treated PC3 cells. Apoptosis and cell death were confirmed by Annexin V and in conjunction with 7-AAD and Muse Cell Analyzer. Triplicate wells containing 1 × 10^4 cells/well were exposed to 0.5, 2.5, 5, 10, and 50 µM concentrations of genistein for 48 hr.

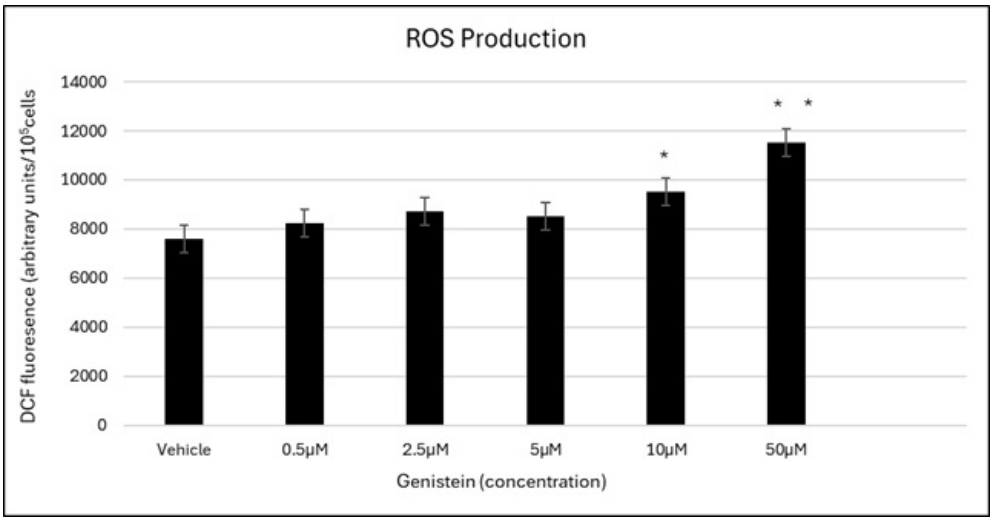

Figure 4. PC3 cells treated with 0.5, 2.5, 5, 10, and 50µM genistein for 1hr were evaluated for ROS production. A significant rise in ROS level was observed at 10 µM ($p < 0.01$), which further increased at 50 µM genistein. Error bars represent the *SEM* values. $p < 0.01$, $p < 0.001$*** represents statistically significant.**

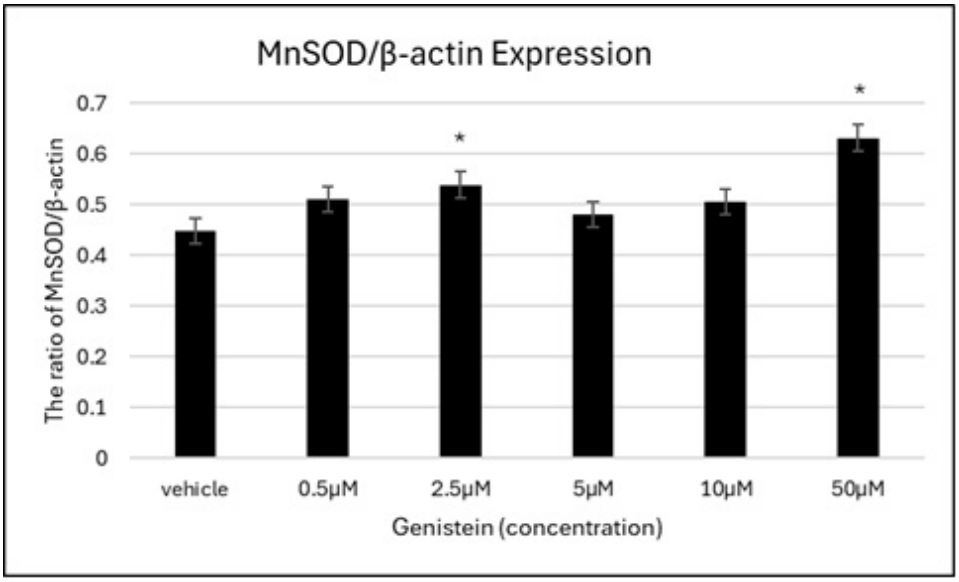

Figure 5. MnSOD protein expression level was determined by Western Blot by treating PC3 cells with 0.5, 2.5, 5, 10, and 50μM genistein for 48hr. Error bars represent the SD values.

p < 0.0001 **represents statistically significant.**

DISCUSSION

In this study, we demonstrated that genistein exhibits multifaceted effects on PC3 androgen-independent prostate cancer cells, impacting cell viability, migration, ROS generation, MnSOD expression, and apoptosis induction in a concentration-dependent manner. Our results suggest a biphasic influence of genistein on the growth of cells, with lower, physiologically relevant concentrations (0.5-10μM) displaying a modest stimulating effect, while higher, pharmacologically achievable concentrations (50 μM) give rise to a time-dependent decline in cell viability and a significant restriction on cell migration. The inhibitory impact on migration correlates with findings that dietary soy intake, abundant in genistein, may be linked with reduced cancer progression in some populations [13].

The mechanism causing these effects likely involves the modulation of ROS generation. Higher genistein concentrations significantly increased ROS generation, which probably contributed to the observed cytotoxicity, but lower concentrations had a minimal impact on ROS levels. This finding supports earlier research showing genistein can cause oxidative stress in malignant cells, which in turn triggers apoptosis [14]. To lessen oxidative damage, cells may respond adaptively to the elevated ROS levels by increasing MnSOD production, as shown at higher genistein concentrations [15]. Increased quantities of ROS generation by higher genistein concentrations, however, probably exceed this antioxidant response's capabilities and cause the cells to eventually undergo apoptosis. This phenomenon is supported by our previous study which demonstrates a dose-specific increase in apoptosis, especially at maximum concentrations (50 μM), where a significant shift towards late apoptosis/cell death was noted [16].

However, it is crucial to remember that the concentration required to induce these effects *in vitro* can be higher than those obtained by dietary consumption alone. The crucial importance of optimal genistein dosage for therapeutic applications is demonstrated by this biphasic response, where lower concentrations promote proliferation, and higher concentrations induce cytotoxic outcomes [16].

These findings align with the literature which exhibits genistein's promising potential in many cancer models regarding suppressing cancer cell progression in various signaling pathways [17]. According to the epidemiological studies, regular soy consumption, a

genistein source, is associated with lower cancer risk and better clinical outcomes in cancer patients [18-19]. Given the crucial role of oxidative stress, inherent in aggressive prostate cancer cells, substances such as genistein could be a potential treatment strategy [20]. Additionally, recent studies indicate that genistein may improve treatment results for aggressive prostate cancer when combined with traditional chemotherapy. By modifying several signaling pathways, including those linked to apoptosis and drug resistance, genistein has been demonstrated to make cancer cells more sensitive to chemotherapy. In particular, genistein can increase the efficacy of chemotherapeutic agents by blocking the NF-κB pathway, which is known to promote chemoresistance [21]. Furthermore, genistein's ROS production may work in concert with chemotherapeutic agents that use oxidative stress to kill cancer cells [11].

These findings suggest that genistein could act as an adjuvant to chemotherapy, particularly in aggressive prostate cancer patients, by amplifying oxidative stress, enhancing apoptosis, and overcoming chemoresistance. This study has some limitations which focus on the PC3 cell line. Although PC3 cells are androgen-independent and exhibit aggressive prostate cancer characteristics, given the heterogeneity of prostate cancer, conducting similar experiments on different cell lines (e.g., LNCaP-Abl [Androgen-Independent Derivative of LNCaP] or castration-resistant prostate cancer cell lines such as C4-2 and C4-2B) enhance the generalizability of genistein's effects. In addition, this study was carried out entirely *in vitro*. We think that genistein bioavailability and metabolism may differ *in vivo*.

In conclusion, our findings imply that genistein, particularly at higher concentrations, inhibits the growth of androgen-independent PC3 prostate cancer cells through processes that include apoptosis induction, MnSOD regulation, and elevated oxidative stress. According to these results, genistein should be further studied as a possible treatment for prostate cancer, especially to target aggressive, androgen-independent forms of the disease. Although further research is needed to fully explore the possibility of synergistic effects with chemotherapy, genistein's biphasic effects and any drug interactions must be carefully considered to maximize its therapeutic potential.

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Ethical Approval: As the study is conducted on the cell line, there is no need for ethical clearance.
Peer-review: Externally peer-reviewed.
Conflict of Interest: Authors declared no conflict of interest.
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REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Ca Cancer J Clin. 2021;71:209–249.
2. Chandrasekar T, Yang JC, Gao AC, Evans CP. Mechanisms of resistance in castration-resistant prostate cancer (CRPC). Transl Androl Urol. 2015;4:365-80.
3. Joshi H, Gupta DS, Abjani NK, Kaur G, Mohan CD, Kaur J et al. Genistein: a promising modulator of apoptosis and survival signaling in cancer. Naunyn Schmiedebergs Arch Pharmacol. 2023;396:2893-2910.
4. Sawada N, Iwasaki M, Yamaji T, Shimazu T, Inoue M, Tsugane S. Japan Public Health Center-based Prospective Study Group. Soy and isoflavone consumption and subsequent risk of prostate cancer mortality: the Japan Public Health Center-based Prospective Study. Int J Epidemiol. 2020;49:1553-1561.
5. Sharifi-Rad J, Quispe C, Imran M, Rauf A, Nadeem M, Gondal TA et al. Genistein: an integrative overview of its mode of action, pharmacological properties, and health benefits. Oxid Med Cell Longev. 2021;2021:3268136.
6. Liu Y, Kyle E, Lieberman R, Crowell J, Kellof G, Bergan RC. Focal adhesion kinase (FAK) phosphorylation is not required for genistein-induced FAK-beta-1-integrin complex formation. Clin Exp Metastasis. 2000;18:203-12.
7. Hörmann V, Kumi-Diaka J, Durity M, Rathinavelu A. Anticancer activities of genistein-topotecan combination in prostate cancer cells. J Cell Mol Med. 2012;16:2631-6.
8. Fu Z, Cao X, Liu L, Cao X, Cui Y, Li X et al. Genistein inhibits lung cancer cell stem-like characteristics by modulating MnSOD and FoxM1 expression. Oncol Lett. 2020;20:2506-2515.
9. Rasheed S, Rehman K, Shahid M, Suhail S, Akash MSH. Therapeutic potentials of genistein: New insights and perspectives. J Food Biochem. 2022;46:e14228.
10. Jordaens L, Arias-Alvarez M, Pintelon I, Thys S, Valckx S, Dezhkam Y et al. Elevated non-esterified fatty acid concentrations hamper bovine oviductal

epithelial cell physiology in three different in vitro culture systems. Theriogenology. 2015;84:899-910.
11. Wynne S, Djakiew D. NSAID inhibition of prostate cancer cell migration is mediated by Nag-1 Induction via the p38 MAPK-p75(NTR) pathway. Mol Cancer Res. 2010;8:1656-64.
12. Goncu B, Sevgi E, Kizilarslan Hancer C, Gokay G, Ozten N. Differential anti-proliferative and apoptotic effects of lichen species on human prostate carcinoma cells. PLoS One. 2020;15:e0244831.
13. Wang C, Ding K, Xie X, Zhou J, Liu P, Wang S et al. Soy product consumption and the risk of cancer: a systematic review and meta-analysis of observational studies. Nutrients. 2024;16:986.
14. Alorda-Clara M, Torrens-Mas M, Morla-Barcelo PM, Roca P, Sastre-Serra J, Pons DG et al. High concentrations of genistein decrease cell viability depending on oxidative stress and inflammation in colon cancer cell lines. Int J Mol Sci. 2022;23:7526.
15. Borrás C, Gambini J, Gómez-Cabrera MC, Sastre J, Pallardó FV, Mann GE et al. Genistein, a soy isoflavone, up-regulates expression of antioxidant genes: involvement of estrogen receptors, ERK1/2, and NFκB. FASEB J. 2006;20:2136-2138.
16. Terzioglu-Usak S, Yildiz MT, Goncu B, Ozten-Kandas N. Achieving the balance: Biphasic effects of genistein on PC-3 cells. J Food Biochem. 2019;43:e12951.
17. Pavese JM, Farmer RL, Bergan RC. Inhibition of cancer cell invasion and metastasis by genistein. Cancer Metastasis Rev. 2010;29:465-82.
18. Messina M. Impact of soy foods on the development of breast cancer and the prognosis of breast cancer patients. Forsch Komplementmed. 2016;23:75-80.
19. Yang G, Shu XO, Li HL, Chow WH, Wen W, Xiang YB, et al. Prediagnosis soy food consumption and lung cancer survival in women. J Clin Oncol. 2013;31:1548-53.
20. Kumar B, Koul S, Khandrika L, Meacham RB, Koul HK. Oxidative stress is inherent in prostate cancer cells and is required for aggressive phenotype. Cancer Res. 2008;68:1777-85.
21. Tuli HS, Tuorkey MJ, Thakral F, Sak K, Kumar M, Sharma AK et al. Molecular mechanisms of action of genistein in cancer: recent advances. Front Pharmacol. 2019;10:1336.