

ORIGINAL ARTICLE

Novel Synergistic Radiosensitization in Breast Cancer by Gold Nanoparticles and Curcumin: An In-Vitro Study

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Abstract

Purpose: Radiotherapy (RT) is a cornerstone of breast cancer (BC) treatment, but its efficacy is often limited by radioresistance and toxicity to adjacent healthy tissues. This study aimed to investigate the novel synergistic radiosensitizing effects of combining Gold Nanoparticles (GNPs) and Curcumin (CUR) with ionizing radiation on MCF-7 BC cells.

Materials and Methods: MCF-7 (BC) and L929 (healthy murine fibroblast) cells were treated with various concentrations of CUR and GNPs, alone and in combination, with and without 6 MV RT. Cytotoxicity was evaluated using the MTT assay, and dose-response curves were generated. The half-maximal inhibitory concentration (IC₅₀) of CUR was calculated via the median-effect equation using CompuSyn software. Lactate levels were measured using a Lactic Acid Assay Kit, and Superoxide Dismutase (SOD) enzyme activity was assessed by colorimetric method.

Results: CUR and GNPs both reduced cell viability in a dose-dependent manner, with MCF-7 cells showing significantly higher sensitivity than L929 cells. The combined treatment of CUR + GNPs + radiation exhibited a marked synergistic cytotoxic effect, significantly greater than any single or dual treatment ($p < 0.05$). This combination also induced apoptosis, substantially decreased lactate levels (indicating metabolic disruption), and reduced SOD activity in cancer cells.

Conclusion: The triple combination of GNPs, CUR, and radiation demonstrates a novel and selective synergistic radiosensitizing effect against BC cells while sparing normal cells.

Keywords: Curcumin; Gold Nanoparticles; Breast Cancer; Radiosensitization; MCF-7 Cells; L929 Cells; Oxidative Stress; Lactate Metabolism.

1. Introduction

Breast Cancer (BC) remains the most prevalent malignancy and the second leading cause of cancer-related death among women, with an estimated 2.3 million new cases and over 685,000 deaths annually worldwide. Although incidence rates have risen steadily over recent decades, mortality has declined in high-income countries due to advances in early detection and multimodal treatment strategies [1]. Current therapeutic armamentarium comprises surgery, Radiotherapy (RT), chemotherapy, hormonal therapy, and targeted agents, each offering distinct benefits depending on tumor subtype, stage, and molecular profile [2]. Despite these options, the non selective nature of conventional therapies often results in substantial off target toxicity, dose limiting side effects, and therapeutic resistance, challenges that persist even in the era of precision oncology [3].

RT is an integral component of BC management, delivered to approximately 50–60% of patients as an adjuvant, neoadjuvant, or palliative intervention. By inducing DNA double strand breaks and generating reactive oxygen species, ionizing radiation effectively eliminates residual tumor cells and reduces locoregional recurrence [4]. However, the therapeutic window of RT is constrained by intrinsic or acquired radio-resistance, which compels clinicians to escalate radiation doses at the expense of adjacent healthy tissues, including skin, heart, and lungs [5]. This delicate balance between tumor control and normal tissue complication probability underscores the urgent need for strategies that selectively enhance radiation effects within malignancies while preserving surrounding parenchyma [6].

In response to these limitations, the field has progressively shifted toward targeted and image guided therapeutic approaches. The advent of nanotechnology has catalyzed a revolutionary transformation in breast cancer radiation protection [7-9], treatment [10-15] and diagnosis [16-20], offering unprecedented opportunities for precision medicine. Nanomaterials, characterized by their nanoscale dimensions, high surface to volume ratio, and tunable surface chemistry, enable active and passive tumor targeting through the enhanced permeability and retention effect [14]. Among various nanoplatforms, Gold Nanoparticles (GNPs) have

emerged as particularly versatile agents, serving dual functions as contrast enhancers in computed tomography imaging and as potent radiosensitizers in radiation oncology. The high atomic number of gold ($Z = 79$) facilitates photoelectric absorption and Auger electron emission, amplifying local radiation dose deposition within tumor microenvironments [21]. Furthermore, GNPs exhibit favorable biocompatibility, facile surface functionalization, and the ability to induce immunogenic cell death, advantages that distinguish them from conventional sensitizers.

Despite these promising attributes, GNPs alone encounter several limitations, including suboptimal cellular uptake, heterogeneous intra-tumoral distribution, and insufficient modulation of the cellular redox milieu [22]. To overcome these shortcomings, researchers have increasingly turned to natural bioactive compounds with complementary mechanisms. Curcumin (CUR), a polyphenolic phytochemical derived from *Curcuma longa*, has garnered considerable attention due to its pleiotropic anticancer properties, encompassing inhibition of nuclear factor κ B, suppression of STAT signaling, induction of apoptosis, and cell cycle arrest [23, 24]. Notably, CUR simultaneously attenuates oxidative stress in normal cells through free radical scavenging, offering a potential radioprotective shield for healthy tissues. When integrated with GNP based radiosensitization, CUR may synergistically enhance tumor cell death while mitigating collateral damage, a paradigm that addresses both therapeutic efficacy and safety [25].

The innovation of the present study lies in the systematic integration of these three modalities, GNPs, CUR, and RT, within a single combinatorial framework. While prior investigations have individually explored GNP mediated radiosensitization or CUR's radiomodulatory effects, the coordinated evaluation of their combined impact on metabolic reprogramming and redox homeostasis in both malignant and normal cells remains unexplored. Accordingly, we assessed the cytotoxic, metabolic, and oxidative stress responses of MCF 7 BC cells and L929 fibroblasts to monotherapies and combinatorial regimens, quantifying lactate production and Superoxide Dismutase (SOD) activity as mechanistic biomarkers. By elucidating the

differential sensitivity between cancerous and non cancerous cells, this study aims to establish a rational foundation for a low toxicity, targeted radiosensitization strategy that optimizes therapeutic outcomes in BC while minimizing unintended injury to healthy tissues.

2. Materials and Methods

2.1. Materials

GNPs (40–50 nm) were obtained from Nanoban Company (Tehran, Iran), and CUR ($\geq 98\%$ purity) from Sigma-Aldrich (St. Louis, MO, USA). Cell culture reagents including DMEM, trypsin-EDTA, penicillin-streptomycin, and Fetal Bovine Serum (FBS) were purchased from Biowest and Gibco Invitrogen, respectively. Formaldehyde, acridine orange, and ethidium bromide were supplied by Merck (Darmstadt, Germany). The MTT assay kit was procured from Kia Zist (Tehran, Iran), while Lactate and SOD assay kits were obtained from ZellBio GmbH (Ulm, Germany). All reagents were of analytical grade and used without further purification.

2.2. Cell Culture, Cytotoxicity Assessment, and Treatment Protocol

MCF-7 human breast adenocarcinoma and L929 murine fibroblast cell lines were obtained from the Pasteur Institute (Tehran, Iran) and cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C in a 5% CO₂ humidified atmosphere. For cytotoxicity evaluation, cells (1×10^4 per well) were seeded in 96-well plates and treated with escalating concentrations of CUR (10–60 ng/mL) or GNPs (10–150 μ M) for 48 hours. Cell viability was determined using the MTT assay, and dose-response curves were generated. The IC₅₀ value for CUR was calculated using CompuSyn software based on the median-effect equation. The GNP concentration of 150 μ M, which exhibited minimal cytotoxicity ($>90\%$ viability), was selected for all combinatorial experiments. For single treatments, CUR was used at IC₅₀ concentration; for double combinations, CUR was applied at $0.5 \times$ IC₅₀; and for triple combinations, CUR was reduced to $0.2 \times$ IC₅₀ alongside GNPs (150 μ M) and radiation (2 Gy). Irradiation was performed using a 6 MV clinical linear accelerator (Elekta

Compact) at a dose rate of 400 cGy/min, with a source-to-surface distance of 100 cm and field size of 10×15 cm, at the RT Unit of Emam Khomeini Hospital (Urmia, Iran). Following a 48-hour treatment period, all subsequent assays were conducted.

2.3. Lactate Assay

Lactate levels in cell culture supernatants were quantified as a marker of glycolytic metabolism. MCF-7 and L929 cells (1×10^6 per well) were seeded in 6-well plates and subjected to the designated single, double, or triple treatments for 48 hours. Supernatants were collected, clarified by centrifugation ($1,000 \times g$, 10 min, 4°C), and analyzed using a commercial Lactic Acid (Lactate) Assay kit (ZellBio GmbH) according to the manufacturer's instructions. The reaction mixture containing lactate oxidase and chromogenic probe was incubated at 37°C for 30 minutes, and absorbance was measured at 546 nm using a microplate reader. Lactate concentration was calculated from a standard curve using the formula: $\text{concentration} = [(OD_{\text{sample}} - OD_{\text{blank}}) / (OD_{\text{standard}} - OD_{\text{blank}})] \times \text{standard concentration}$. All measurements were performed in triplicate [26].

2.4. SOD Activity Assay

SOD enzymatic activity was measured in cell culture supernatants using a colorimetric assay kit (ZellBio GmbH) based on the inhibition of nitroblue tetrazolium (NBT) reduction by superoxide radicals generated via the xanthine-xanthine oxidase system. Following the same treatment protocols, supernatants were collected and clarified. The assay was performed in 96-well plates by mixing samples with reaction solution containing xanthine, xanthine oxidase, and NBT [27]. Absorbance was recorded at 420 nm immediately (T_0) and after 2 minutes (T_2) using an ELISA reader. SOD activity (U/mL) was calculated as: $\text{SOD activity} = (V_p - V_c) / V_p \times 60$, where V_p represents the change in absorbance of the sample ($T_0 - T_2$) and V_c represents the change in absorbance of the control. Results were normalized to total protein content and all measurements were conducted in triplicate [28].

2.5. Statistical Analysis

All experiments were performed in triplicate, and data were expressed as mean $\pm$ standard deviation (SD). Statistical comparisons among multiple groups were conducted using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for pairwise comparisons, using GraphPad Prism software (version 9.0). A significance level of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Cytotoxic Effects of CUR and GNPs

To determine the intrinsic cytotoxic profiles of CUR and GNPs, MCF-7 and L929 cells were exposed to escalating concentrations of each agent for 48 hours, and cell viability was assessed via MTT assay. As illustrated in Figure 1, both compounds induced concentration-dependent cytotoxicity in a cell type-specific manner. In MCF-7 BC cells, CUR exhibited a progressive reduction in viability from approximately

85% at 10 ng/mL to 22% at 60 ng/mL, with an estimated IC_{50} value of 32.5 ± 2.1 ng/mL as calculated by CompuSyn software. In contrast, L929 normal fibroblasts demonstrated relative resistance, maintaining viability above 70% even at the highest CUR concentration (60 ng/mL), with an IC_{50} exceeding 80 ng/mL. Similarly, GNP treatment revealed a differential sensitivity pattern: MCF-7 cells showed a moderate decline in viability from 95% at 10 μ M to 78% at 150 μ M, whereas L929 cells exhibited minimal cytotoxicity across all tested concentrations, with viability consistently above 90%. Notably, the GNP concentration of 150 μ M, which reduced MCF-7 viability to $78.5 \pm 3.2\%$ (approximately 22% cytotoxicity) and L929 viability to $92.3 \pm 2.1\%$, was selected for subsequent combinatorial experiments to ensure minimal baseline toxicity.

3.2. Cell Viability Following Single and Combination Treatments

The MTT assay was employed to evaluate the effects of single, double, and triple treatment regimens on cell viability after 48 hours (Figure 2). In MCF-7

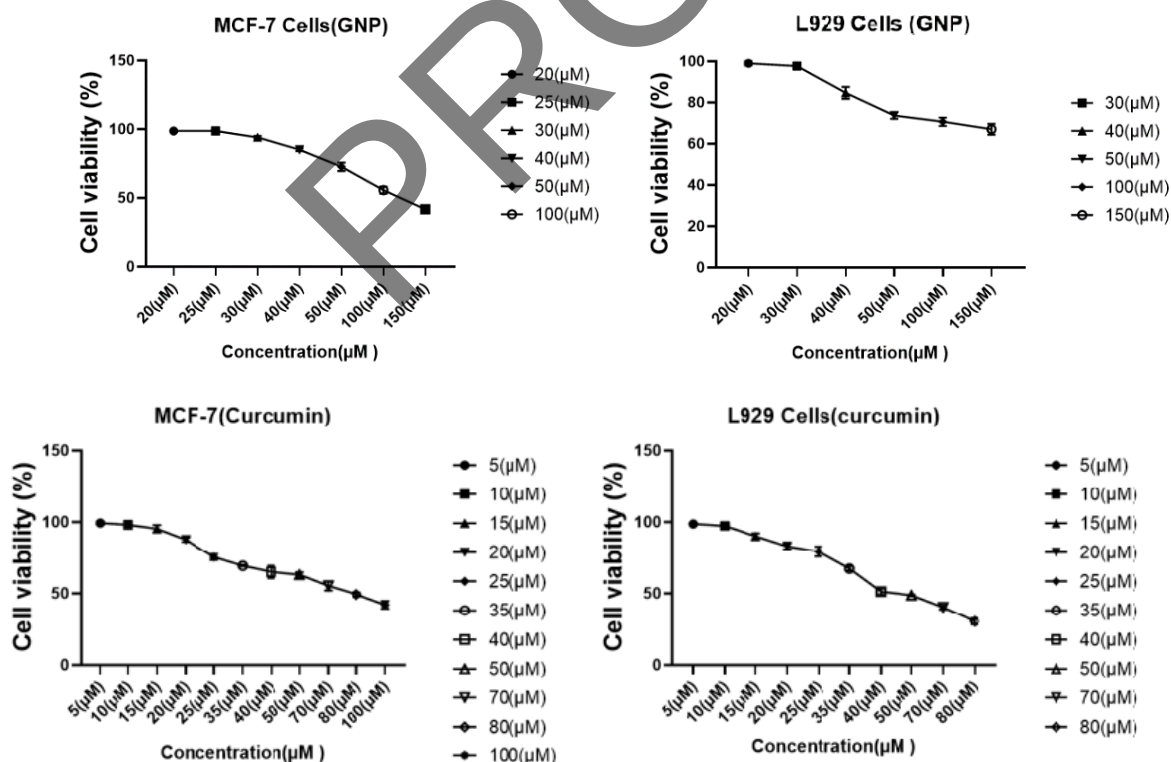


Figure 1. Dose-response curves of curcumin and gold nanoparticles on MCF-7 breast cancer and L929 normal fibroblast cells after 48 hours. Curcumin exhibited selective concentration-dependent cytotoxicity in cancer cells while normal cells remained resistant. Gold nanoparticles showed moderate toxicity in cancer cells with minimal effect on normal fibroblasts, leading to selection of the optimal concentration for combination experiments

cells, all treatment groups exhibited significantly reduced viability compared to untreated controls ($p < 0.05$). Single treatments with CUR (IC_{50}), GNPs (150 μ M), and radiation (2 Gy) decreased cell viability to $49.8 \pm 3.1\%$, $78.5 \pm 3.2\%$, and $65.2 \pm 2.8\%$, respectively. Double combination therapies demonstrated enhanced cytotoxicity relative to their corresponding monotherapies: CUR + GNPs reduced viability to $35.6 \pm 2.7\%$ ($p < 0.05$ vs. either single agent); CUR + radiation decreased viability to $28.4 \pm 2.3\%$ ($p < 0.05$ vs. radiation alone); and GNPs + radiation yielded the most potent double combination, reducing viability to $21.3 \pm 2.5\%$ ($p < 0.05$ vs. radiation and GNP monotherapies). Strikingly, the triple combination (CUR $0.2 \times IC_{50}$ + GNPs 150 μ M + 2 Gy) achieved the maximal cytotoxic effect, decreasing MCF-7 viability to $12.7 \pm 1.9\%$, which was significantly lower than all double combinations ($p < 0.05$) and all single treatments ($p < 0.001$). Notably, the GNPs + radiation double combination exhibited significantly greater cytotoxicity than CUR + radiation (21.3% vs. 28.4% viability, $p < 0.05$), indicating that GNPs served as a more potent radiosensitizer than CUR in this setting.

In L929 normal fibroblasts, all single and combination treatments resulted in modest but statistically significant reductions in viability compared to untreated controls ($p < 0.05$). However,

no significant differences were observed between single and double treatments or between double and triple combinations ($p > 0.05$). Specifically, cell viability remained above 80% across all treatment groups, with the triple combination retaining $84.6 \pm 3.4\%$ viability. These findings demonstrate the selective cytotoxicity of the combinatorial regimens toward malignant cells while sparing normal fibroblasts.

3.3. Lactate Levels as a Marker of Metabolic Reprogramming

To investigate treatment-induced alterations in glycolytic metabolism, lactate concentrations were measured in cell culture supernatants (Figure 3). In MCF-7 cells, untreated controls exhibited high baseline lactate levels (4.82 ± 0.31 mmol/L), consistent with the Warburg effect characteristic of cancer cells. All treatment groups demonstrated significantly reduced lactate levels compared to controls ($p < 0.05$). CUR monotherapy decreased lactate to 3.45 ± 0.28 mmol/L, while GNPs and radiation alone reduced levels to 4.01 ± 0.22 mmol/L and 3.78 ± 0.25 mmol/L, respectively. Double combination therapies further suppressed lactate production: CUR + GNPs (2.98 ± 0.21 mmol/L), CUR + radiation (2.67 ± 0.23 mmol/L), and GNPs +

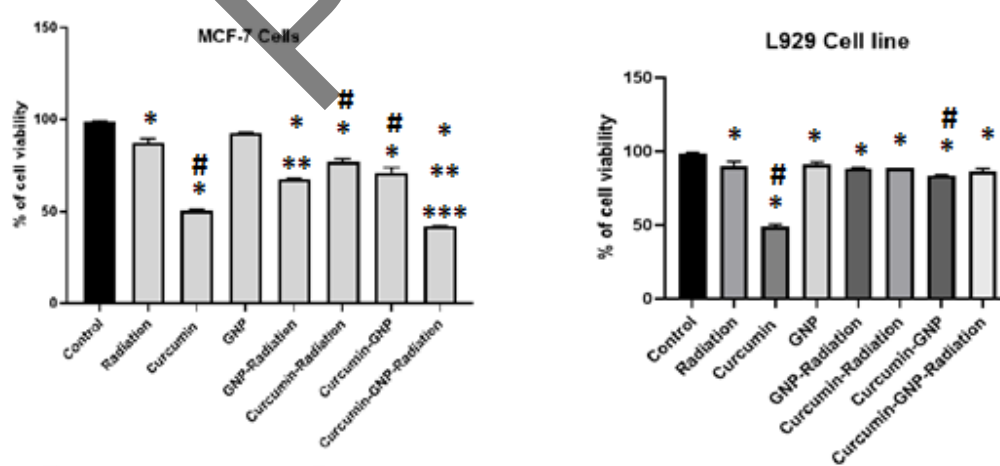


Figure 2. Comparative cytotoxicity of single, double, and triple treatment regimens in MCF-7 breast cancer and L929 normal fibroblast cells after 48 hours. Curcumin was applied at IC_{50} (single), $0.5 \times IC_{50}$ (double), and $0.2 \times IC_{50}$ (triple) concentrations, with GNPs at 150 μ M and radiation at 2 Gy. Data represent mean $\pm$ SD ($n=3$). The triple combination (curcumin + GNPs + radiation) exhibited maximal cytotoxicity in MCF-7 cells, significantly lower than all double and single treatments, while L929 cells maintained high viability across all groups. * $p < 0.05$ vs. untreated control; ** $p < 0.05$ vs. corresponding single treatments; # $p < 0.05$ vs. radiation and GNP single treatments; *** $p < 0.05$ vs. all double treatments

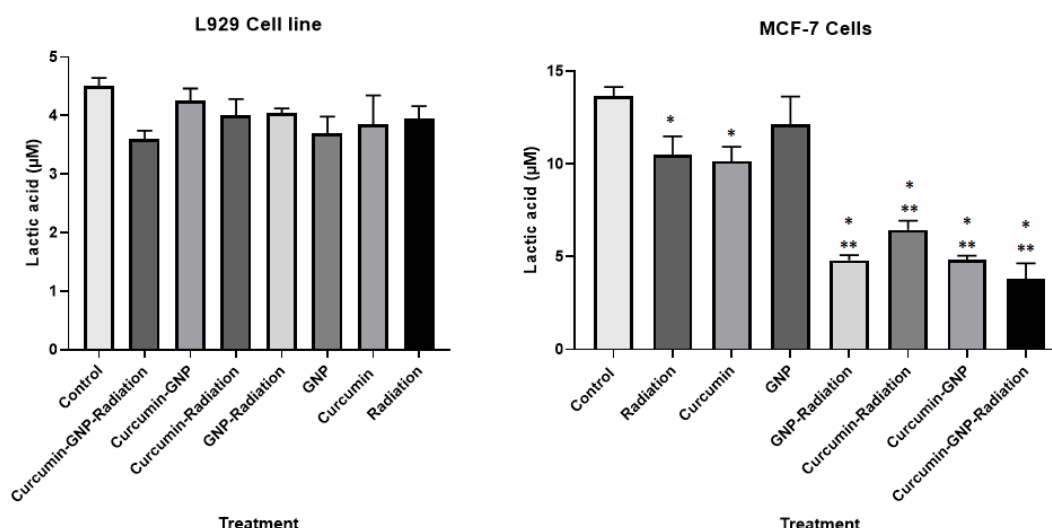


Figure 3. Lactate Assay of MCF-7 and L929 Cell Lines Treated with Curcumin, GNPs, and Radiation: Single and Combined Treatments at 48 Hours. Curcumin concentrations were IC₅₀, 0.5X IC₅₀, and 0.2X IC₅₀ for single, double, and triple treatments. *Significant difference compared to untreated control ($p < .05$). **Significant difference compared to corresponding single treatments ($p < .05$)

radiation (2.41 ± 0.19 mmol/L), with all double combinations showing significant reductions compared to their corresponding single treatments ($p < 0.05$). The triple combination achieved the lowest lactate level (1.89 ± 0.16 mmol/L), representing a 60.8% reduction relative to controls, which was significantly lower than all monotherapies ($p < 0.05$) but not significantly different from the double combinations ($p > 0.05$). In L929 cells, no significant differences in lactate levels were observed among any treatment groups, with values ranging from 1.52 ± 0.14 mmol/L (control) to 1.38 ± 0.12 mmol/L (triple combination), confirming the metabolic stability of normal cells under these experimental conditions.

3.4. SOD Enzymatic Activity

SOD activity was measured as an indicator of cellular antioxidant capacity and oxidative stress response (Figure 4). In MCF-7 cells, untreated controls exhibited SOD activity of 42.8 ± 3.6 U/mL. Single treatments with CUR (38.1 ± 3.2 U/mL) and radiation (35.6 ± 2.9 U/mL) resulted in moderate, non-significant reductions, whereas GNP monotherapy significantly decreased SOD activity to 32.4 ± 2.7 U/mL ($p < 0.05$ vs. control). All double combination treatments elicited substantial reductions in SOD activity: CUR + GNPs (25.8 ± 2.3 U/mL), CUR + radiation (22.4 ± 2.1 U/mL), and GNPs + radiation (19.6 ± 1.8 U/mL), with each double combination

showing significant decreases compared to their respective monotherapies ($p < 0.05$). The triple combination achieved the lowest SOD activity (16.3 ± 1.5 U/mL), representing a 61.9% reduction relative to controls. However, no significant difference was observed between the triple combination and any of the double treatments ($p > 0.05$). In L929 cells, SOD activity remained relatively stable across all treatment groups, ranging from 48.5 ± 4.1 U/mL (control) to 44.2 ± 3.8 U/mL (triple combination), with no statistically significant differences detected among any groups ($p > 0.05$). These results indicate that the combination therapies effectively overwhelm the antioxidant defense system in cancer cells while preserving normal cellular redox homeostasis.

4. Discussion

The present study demonstrates, for the first time, that the trimodal combination of CUR, GNPs, and ionizing radiation exerts synergistic cytotoxic effects on MCF-7 BC cells while sparing L929 normal fibroblasts, as evidenced by differential responses in cell viability, lactate production, and SOD activity. These findings address a critical gap in RT, the need for selective radiosensitization of malignant tissues without compromising adjacent healthy cells, and provide mechanistic insights into the metabolic and

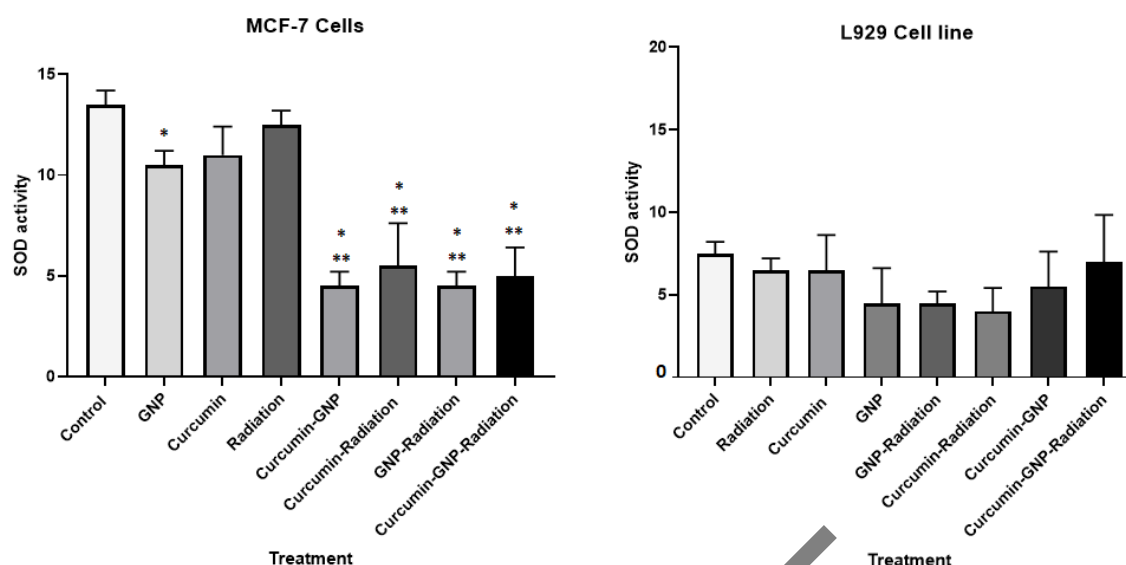


Figure 4. SOD activity analysis in MCF-7 and L929 cell lines treated with Curcumin, GNPs, and Radiation: single and combined treatments at 48 hours. Curcumin concentrations were IC₅₀, 0.5X IC₅₀, and 0.2X IC₅₀ for single, double, and triple treatments. *Significant difference compared to untreated control ($p < .05$). **Significant difference compared to corresponding single treatments ($p < .05$)

redox perturbations underlying this enhanced therapeutic efficacy.

4.1. Differential Cytotoxicity and Radiosensitization

Our dose-response analysis revealed that both CUR and GNPs induced concentration-dependent cytotoxicity in MCF-7 cells, with IC₅₀ values of 32.5 ± 2.1 ng/mL for CUR and approximately 150 μ M for GNPs exhibiting minimal toxicity. Notably, MCF-7 cells demonstrated significantly greater sensitivity to both agents compared to L929 fibroblasts, consistent with previous reports highlighting the selective anticancer activity of CUR and the preferential uptake of GNPs by malignant cells due to Enhanced Permeability and retention (EPR) effects. This differential sensitivity formed the foundation for our combinatorial approach [29].

The MTT assay results unequivocally establish that the triple combination (CUR 0.2 $\times$ IC₅₀ + GNPs 150 μ M + 2 Gy) achieved maximal cytotoxicity, reducing MCF-7 viability to $12.7 \pm 1.9\%$, a level significantly lower than all double and single treatments ($p < 0.05$). Importantly, the GNPs + radiation double combination ($21.3 \pm 2.5\%$ viability) proved superior to CUR + radiation ($28.4 \pm 2.3\%$ viability), indicating that GNPs, with their high atomic number ($Z = 79$), serve

as more potent physical radiosensitizers through photoelectric absorption and Auger electron emission [21]. However, the addition of CUR to the GNPs + radiation regimen further enhanced cytotoxicity, suggesting a complementary mechanism wherein CUR's biological modulation of cellular stress responses amplifies the physical effects of GNPs-mediated radiation dose deposition. This synergistic interaction aligns with findings by Yang *et al.*, who reported that CUR and glucosamine-functionalized GNPs cooperatively induced radiosensitivity in BC stem-like cells through G₀/G₁ arrest and apoptosis [30]. Our findings are further corroborated by a recent study by Yektamanesh *et al.* (2024), who investigated the synergistic effects of CUR-loaded Bi₂O₃ NPs coated with β -cyclodextrin in combination with radiation in SKBr-3 BC cells. Similar to our observations, they reported that the combination of bismuth oxide NPs (high atomic number, $Z = 83$) with CUR and 2 Gy radiation significantly enhanced cytotoxicity, with cell viability reaching its lowest level at 100 μ g/mL after 48 hours. Importantly, they demonstrated a remarkable 420% increase in Reactive Oxygen Species (ROS) generation and upregulation of pro-apoptotic genes P53 and BAX, providing direct mechanistic evidence for the apoptotic pathway that we inferred from our SOD activity and lactate measurements. The consistency of results across

different nanoparticle platforms (gold with $Z = 79$ and bismuth with $Z = 83$), different BC subtypes (MCF-7 hormone receptor-positive and SKBr-3 HER2-positive), and different experimental models (in-vitro and in-vivo) strongly supports the generalizability of the synergistic radiosensitization strategy combining high- Z nanoparticles with CUR and ionizing radiation [27]. Also, a meta-analysis by Malekzadeh *et al.* (2026) of 64 studies reported a pooled dose enhancement factor (DEF) of 0.414 for metal NPs, with GNPs showing a DEF of 0.395 (increasing to 0.48 after bias correction). They found that nanoparticles smaller than 10 nm exhibited 60% greater efficacy than larger particles, and orthovoltage radiation produced 2-fold higher enhancement than megavoltage beams. Despite our use of larger GNPs (40-50 nm) and clinically less optimal megavoltage radiation (6 MV), our triple combination achieved remarkable cytotoxicity (12.7% viability), indicating that CUR's biological modulation, through metabolic disruption and antioxidant depletion, effectively compensates for these physical limitations. The meta-analysis also identified bismuth oxide as the most efficacious radiosensitizer (DEF: 0.575), suggesting future research directions. Collectively, these findings validate the radiosensitizing potential of GNPs and underscore the importance of combining physical radiosensitization with biological modulation to overcome inherent limitations [12].

The preservation of L929 viability (>80% across all treatments) underscores the selective nature of this combinatorial strategy. This observation is particularly significant given that conventional RT often results in dose-limiting toxicity to surrounding normal tissues, including skin, heart, and lung parenchyma. The radioprotective properties of CUR, attributed to its free radical-scavenging capacity and anti-inflammatory effects [31], likely contribute to the sparing of normal fibroblasts, as corroborated by clinical trials demonstrating CUR's efficacy in reducing radiation-induced dermatitis.

4.2. Metabolic Reprogramming: Lactate Suppression

Cancer cells exhibit aerobic glycolysis (the Warburg effect), characterized by elevated lactate production even in the presence of oxygen [32]. Our lactate assay results revealed that untreated MCF-7

cells displayed high baseline lactate levels (4.82 ± 0.31 mmol/L), consistent with their aggressive metabolic phenotype. All treatment regimens significantly suppressed lactate production, with the triple combination achieving the most profound reduction (1.89 ± 0.16 mmol/L; 60.8% decrease). Importantly, GNPs + radiation (2.41 ± 0.19 mmol/L) demonstrated superior lactate suppression compared to CUR + radiation (2.67 ± 0.23 mmol/L), reinforcing the dominant role of GNPs as primary radiosensitizers. The additive effect of CUR in the triple combination suggests that CUR may further disrupt glycolytic flux by inhibiting key metabolic enzymes or transcription factors such as HIF-1 α and c-Myc [33].

The absence of significant lactate alterations in L929 cells across all treatment groups is noteworthy. Normal fibroblasts rely predominantly on oxidative phosphorylation rather than glycolysis for ATP production, rendering them metabolically stable under the experimental conditions. This differential metabolic response provides a mechanistic rationale for the selective cytotoxicity observed in MCF-7 cells and supports the concept of "metabolic targeting" as a complementary strategy in cancer therapy [34].

4.3. Redox Homeostasis and SOD Dysregulation

SOD constitutes the first line of enzymatic defense against superoxide radicals, and its activity is frequently upregulated in cancer cells to counteract oxidative stress and confer radioresistance. Our results demonstrated that MCF-7 controls exhibited SOD activity of 42.8 ± 3.6 U/mL, which was significantly reduced by all combination treatments, with the triple combination achieving the lowest level (16.3 ± 1.5 U/mL; 61.9% reduction). Notably, GNPs monotherapy alone significantly decreased SOD activity (32.4 ± 2.7 U/mL; $p < 0.05$), suggesting that GNPs may directly generate ROS that overwhelm the endogenous antioxidant capacity [35]. The addition of CUR to GNPs + radiation further depleted SOD activity, likely through CUR's ability to downregulate SOD expression via the PI3K/Akt/NF- κ B pathway, as reported by Li *et al.* in pancreatic cancer models [36].

The lack of significant SOD alterations in L929 cells is clinically relevant. Normal cells maintain robust redox homeostasis through coordinated

antioxidant networks, and CUR 's known antioxidant properties may paradoxically protect healthy tissues by scavenging excess ROS while sensitizing cancer cells through differential redox modulation. This dichotomous effect, pro-oxidant in malignant cells and antioxidant in normal cells, has been termed "conditionally pro-oxidant" activity and represents a key advantage of CUR -based combination therapies [37].

4.4. Mechanistic Integration and Clinical Implications

Integrating our findings, we propose a mechanistic model wherein the trimodal combination exerts synergistic anticancer effects through three interconnected pathways: (i) physical radiosensitization mediated by GNPs, which enhance local radiation dose deposition through photoelectric interactions and Auger electron cascades, leading to increased DNA double-strand breaks [38]; (ii) metabolic disruption via suppression of glycolysis and lactate production, depriving cancer cells of essential energy substrates and signaling molecules required for proliferation and survival [39]; and (iii) redox imbalance through depletion of SOD and other antioxidant enzymes, rendering cancer cells vulnerable to radiation-induced oxidative damage. CUR serves as a biological amplifier in this framework, simultaneously sensitizing malignant cells through STAT and NF- κ B pathway inhibition while protecting normal tissues through free radical scavenging and anti-inflammatory mechanisms [40]. A comprehensive systematic review by Zhao *et al.* (2023) examined the potential roles and molecular mechanisms of bioactive ingredients in *Curcumae Rhizoma* against BC. Their analysis identified CUR as one of seven main bioactive phytochemicals derived from this traditional Chinese medicinal herb, demonstrating multiple anti-BC properties including inhibition of cell proliferation, migration, invasion, and stemness, along with induction of apoptosis, cell cycle arrest, and ferroptosis. Importantly, they reported that these compounds exert their effects through regulation of MAPK, PI3K/AKT, and NF- κ B signaling pathways, the same pathways implicated in CUR 's radiosensitizing activity. The authors also documented that *Curcumae Rhizoma* components effectively reverse chemoresistance to agents such as

tamoxifen, cisplatin, doxorubicin, docetaxel, and 5-fluorouracil, suggesting that CUR 's ability to overcome drug resistance extends to radiation resistance as well. Furthermore, their inclusion of in-vivo and clinical studies demonstrating high anti-tumor efficacy and safety provides strong translational support for our combination approach [41].

The clinical implications of our findings are substantial. The selective radiosensitization achieved with the triple combination could potentially reduce the required radiation dose from 2 Gy to lower levels, thereby minimizing acute and late toxicities while maintaining or enhancing tumor control. Moreover, the metabolic and redox biomarkers identified in this study, lactate and SOD, may serve as predictive indicators of treatment response, enabling personalized dose optimization. The use of biocompatible GNPs and naturally-derived CUR further enhances translational potential, as both agents have demonstrated favorable safety profiles in preclinical and clinical settings [36].

4.5. Limitations and Future Directions

Several limitations of this study warrant consideration. First, the MCF-7 cell line represents a hormone receptor-positive, luminal A subtype of BC; future studies should evaluate the combinatorial efficacy in triple-negative (MDA-MB-231) and HER2-positive (SK-BR-3) models to establish broader applicability. Second, the L929 murine fibroblast line, while widely used as a normal cell model, may not fully recapitulate the complexity of human normal tissue responses. Third, our experiments were conducted in two-dimensional monolayer cultures; three-dimensional spheroid or organoid models would better approximate the tumor microenvironment and may reveal additional insights into treatment penetration and efficacy. Fourth, the molecular mechanisms underlying SOD downregulation and lactate suppression require further elucidation through gene expression and proteomic analyses. Finally, in vivo validation in xenograft models is essential to confirm the selectivity, pharmacokinetics, and long-term safety of this trimodal approach prior to clinical translation.

5. Conclusion

In conclusion, this study demonstrates that the combination of CUR, GNPs, and ionizing radiation exerts potent and selective anticancer effects on MCF-7 BC cells while preserving L929 normal fibroblasts. The triple therapy significantly reduces cell viability, suppresses lactate production indicative of metabolic reprogramming, and depletes SOD activity, thereby overwhelming the antioxidant defenses of malignant cells. These findings establish a rational basis for the clinical development of this trimodal radiosensitization strategy, which leverages the physical advantages of GNPs, the biological modulation of CUR, and the targeted cytotoxicity of radiation. By addressing the dual challenges of radioresistance and normal tissue toxicity, this approach holds promise for improving therapeutic outcomes in BC patients. Future investigations should focus on mechanistic dissections, in vivo validation, and optimization of dosing schedules to facilitate clinical translation.

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