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RESEARCH ARTICLE

Copper Nanoparticles as a Chemosensitizer: Enhancing Bleomycin Cytotoxicity Against MCF-7 Breast Cancer Cells

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ABSTRACT

Breast cancer, particularly hormone receptor-positive subtypes like MCF-7, remains a major clinical challenge due to chemoresistance, necessitating the development of more effective therapeutic strategies. Copper nanoparticles (CuNPs) have emerged as promising agents in cancer therapy due to their unique physicochemical properties, including oxidative stress induction and enhanced drug delivery. This study investigates the chemosensitizing effects of Nano-Cu in combination with Bleomycin on MCF-7 breast cancer cells. The efficacy of the combination treatment was evaluated by assessing cell viability using the MTT assay. Oxidative stress was quantified by measuring reactive oxygen species (ROS) levels with the DCFH reagent, while lipid peroxidation (LPO) was analyzed by detecting malondialdehyde (MDA) levels. The antioxidant defense system was assessed by measuring glutathione (GSH) levels. The results demonstrated that the combination of CuNPs and Bleomycin significantly reduced MCF-7 cell viability, suggesting potent cytotoxic effects. Additionally, the treatment induced oxidative stress, as evidenced by increased ROS production, elevated MDA levels, and reduced GSH levels. These findings highlight the potential of CuNPs as a chemosensitizer that enhances the efficacy of Bleomycin by promoting oxidative stress and inhibiting MCF-7 breast cancer cell proliferation. This study underscores the therapeutic potential of CuNPs in overcoming chemoresistance and improving breast cancer treatment outcomes.

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INTRODUCTION

Breast cancer is one of the most commonly diagnosed cancers worldwide, accounting for a significant proportion of cancer-related mortality among women(1). Despite advancements in early detection and treatment, breast cancer remains a major health burden, with hormone receptor-positive subtypes, such as MCF-7, exhibiting variable responses to chemotherapy(2). Conventional chemotherapeutic agents, including anthracyclines like Bleomycin, have been widely used to manage breast cancer due to their potent cytotoxic effects(3). However, the clinical utility of Bleomycin is often limited by multiple factors, including the development of multidrug resistance (MDR), systemic toxicity, and reduced therapeutic efficacy over time. These limitations necessitate the exploration of novel strategies to enhance the effectiveness of Bleomycin while minimizing its adverse effects(4, 5).

Bleomycin exerts its anticancer activity through

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multiple mechanisms, including DNA intercalation, inhibition of topoisomerase II, and the generation of reactive oxygen species (ROS), ultimately leading to apoptosis in cancer cells(6). However, breast cancer cells, particularly MCF-7, have been shown to develop resistance to Bleomycin through various mechanisms, such as increased expression of ATP-binding cassette (ABC) transporters, elevated antioxidant defense mechanisms, and enhanced DNA repair pathways(7, 8). These adaptive responses reduce drug accumulation in cancer cells, neutralize ROS-mediated cytotoxicity, and enable tumor cells to evade apoptosis. Consequently, overcoming Bleomycin resistance remains a critical challenge in breast cancer therapy(9).

Nanotechnology has emerged as a promising approach for enhancing the efficacy of chemotherapeutic agents by improving drug delivery, increasing intracellular drug retention, and modulating tumor microenvironmental factors(10). Among the various nanoparticles explored for cancer therapy, metal-based nanoparticles have attracted considerable attention due to their unique physicochemical properties and ability to induce oxidative stress selectively in cancer cells. Copper nanoparticles (CuNPs) have demonstrated significant potential in this regard, as they can generate ROS, disrupt mitochondrial function, and interfere with cellular redox homeostasis(11). These properties make CuNPs an attractive candidate for use as a chemosensitizer to enhance the therapeutic effects of conventional anticancer drugs(12, 13).

Copper is an essential trace element that plays a crucial role in various biological processes, including cellular respiration, antioxidant defense, and enzyme activation(14). However, dysregulated copper homeostasis has been implicated in cancer progression, with elevated intracellular copper levels observed in several malignancies. Cancer cells exhibit increased demand for copper due to its involvement in angiogenesis, metastasis, and redox regulation(15). This differential copper metabolism provides an opportunity to exploit CuNPs as a targeted therapeutic agent that selectively enhances oxidative stress in cancer cells while sparing normal tissues. Previous studies have demonstrated that CuNPs can potentiate the effects of chemotherapy by promoting ROS generation, impairing DNA repair mechanisms, and sensitizing cancer cells to apoptosis(16).

Oxidative stress plays a dual role in cancer therapy, as moderate levels of ROS can promote tumor survival and drug resistance, whereas excessive ROS accumulation can trigger cell death. Many cancer cells, including MCF-7, maintain a delicate balance between ROS production and antioxidant defense mechanisms to sustain their survival(17, 18). The primary intracellular antioxidant, glutathione (GSH), serves as a critical defense mechanism against chemotherapy-induced oxidative damage(19). However, the depletion of GSH disrupts this balance, rendering cancer cells more vulnerable to oxidative stress and apoptosis. CuNPs has been shown to deplete GSH levels while simultaneously increasing lipid peroxidation markers such as malondialdehyde (MDA), leading to enhanced chemosensitivity(20, 21).

Furthermore, mitochondrial dysfunction is a key determinant of chemotherapy response, as mitochondria play a central role in energy metabolism, ROS production, and apoptosis regulation(22). CuNPs has been reported to induce mitochondrial membrane depolarization, leading to cytochrome c release and activation of caspase-dependent apoptotic pathways(23). By impairing mitochondrial function, CuNPs can further amplify the cytotoxic effects of Bleomycin, thereby enhancing treatment efficacy in breast cancer cells(24).

Given these promising properties of Bleomycin, the present study aims to investigate its potential role as a chemosensitizer in enhancing the cytotoxicity of Bleomycin against MCF-7 breast cancer cells. Specifically, we seek to evaluate the combined effects of CuNPs and Bleomycin on cell viability, oxidative stress markers, and apoptotic pathways. By elucidating the underlying mechanisms of action, this study aims to provide valuable insights into the potential application of CuNPs in overcoming chemoresistance and improving breast cancer treatment strategies.

MATERIALS AND METHODS

Chemicals

CuNPs with a size range of 30–10 nm and a purity of 99.99% were sourced from US Research Nanomaterials Inc. and obtained through the local representative, Pishgaman Nano Materials Iranian Company in Mashhad. Bleomycin was purchased from Pfizer (New York, NY, USA). Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin, trypsin-EDTA, and phosphate-buffered saline

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(PBS) were supplied by Gibco (Thermo Fisher Scientific, Waltham, MA, USA). The MTT reagent, 2',7'-dichlorofluorescin diacetate (DCFH-DA), thiobarbituric acid (TBA), and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) were obtained from Sigma-Aldrich. All other chemicals used were of analytical grade and were acquired from standard suppliers.

Cell Culture

MCF-7 human breast cancer cells were obtained from National Cell Bank of Iran (Pasteur Institute, Tehran, Iran) and cultured in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and 1% L-glutamine. The cells were maintained in a humidified atmosphere at 37°C with 5% CO_2. The culture medium was replaced every two to three days, and the cells were passaged using trypsin-EDTA (0.25%) when they reached 80–90% confluence. For experimental procedures, the cells were plated in 96-well plates for viability assays. All experiments were performed at least in triplicate to ensure reproducibility and accuracy of results(25, 26).

Treatment Protocol

MCF-7 cells were seeded in 96-well plates at a density of 1×10^4 cells per well and allowed to attach overnight. Cells were treated with various concentrations of CuNPs (10, 15, 20, 25 and 30 µM) in combination with Bleomycin (10 µg/mL) for 48 hours. Untreated cells served as controls.

Cell Viability Assay

The cytotoxic effects of the treatments on MCF-7 cells were evaluated using the MTT assay. Following treatment, 20 µL of MTT reagent (5 mg/mL) was added to each well, and the cells were incubated for 4 hours at 37°C. The formazan crystals produced during the reaction were dissolved in 150 µL of dimethyl sulfoxide (DMSO). The absorbance was then measured at 570 nm using a microplate reader (BioTek, Winooski, VT, USA). The cell viability percentage was calculated by comparing the absorbance of treated cells with that of the untreated control cells(27, 28).

Assessment of Intracellular ROS Levels

The intracellular reactive oxygen species (ROS) levels were evaluated using the DCFH-DA probe. Cells were incubated with 10 µM DCFH-DA for 30 minutes at 37°C, followed by exposure to CuNPs and **Bleomycin**. Fluorescence intensity was measured using a fluorescence microplate reader (excitation: 485 nm; emission: 530 nm). ROS levels were expressed as a fold change relative to the untreated control group(29, 30).

Determination of Lipid Peroxidation

Lipid peroxidation was assessed by quantifying malondialdehyde (MDA) levels using the thiobarbituric acid-reactive substances (TBARS) assay. Cell lysates were combined with TBA reagent and incubated at 95°C for 30 minutes. After cooling, the samples were centrifuged at 10,000 × g for 10 minutes, and the absorbance of the supernatant was measured at 532 nm. The MDA levels were expressed in nmol/mg protein(31, 32).

Quantification of Glutathione (GSH) Levels

Glutathione levels were measured using the DTNB reagent. Cell lysates were prepared, and 50 µL of each sample was mixed with an equal volume of 10 mM DTNB reagent in PBS. Absorbance was recorded at 412 nm, and GSH concentrations were determined using a standard curve(33, 34).

Statistical Analysis

All experiments were performed in triplicate, and results are presented as the mean ± standard deviation (SD). Statistical analysis was carried out using GraphPad Prism v8.0 (GraphPad Software, San Diego, CA, USA). Comparisons between groups were made using one-way ANOVA followed by Tukey's post hoc test. A p-value of less than 0.05 was considered statistically significant(35, 36).

RESULTS

Effect of CuNPs and Bleomycin on Breast Cancer Cell Viability

The cytotoxic effects of CuNPs in combination with Bleomycin on breast cancer cells (MCF-7) were assessed using the MTT assay. As illustrated in Figure 1, CuNPs at concentrations of 10, 15, 20, 25, and 30 µM, combined with Bleomycin (10 µg/mL), resulted in a dose-dependent decline in cell viability following 48 hours of treatment. The combination therapy exhibited a synergistic effect, leading to significantly greater cytotoxicity compared to either treatment alone ($p<0.05$).

Induction of Oxidative Stress

To evaluate oxidative stress, ROS levels were

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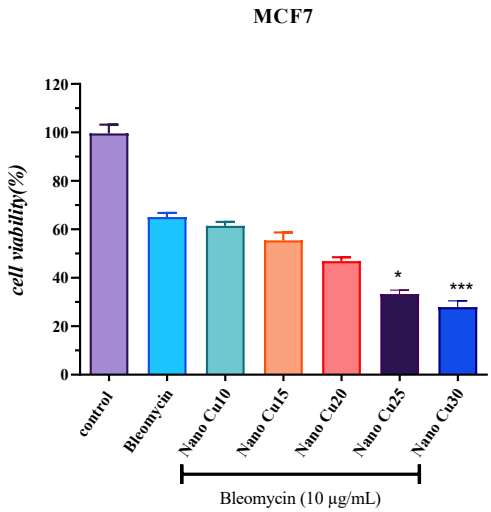

Fig. 2. Dose-dependent decrease in MCF-7 breast cancer cell viability after 24-hour treatment with CuNPs at concentrations of 10, 15, 20, 25, and 30 µM, in combination with 10 µg/mL Bleomycin. The combination treatment significantly enhances cytotoxicity compared to individual treatments. * (p<0.05) and *** (p<0.001) versus Bleomycin group.

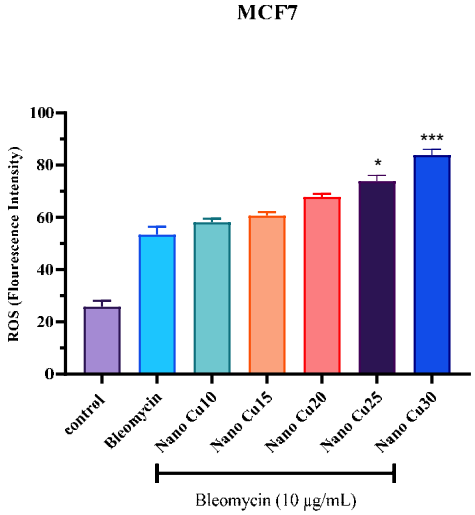

Fig. 3. Dose-dependent increase in ROS levels in MCF-7 breast cancer cells after 48-hour treatment with CuNPs at concentrations of 10, 15, 20, 25, and 30 µM, in combination with 10 µg/mL Bleomycin. The combination therapy significantly elevates ROS levels compared to individual treatments (p<0.05), indicating a synergistic effect. * (p<0.05) and ** (p<0.01) versus Bleomycin group.

measured following treatment with CuNPs in combination with Bleomycin. Treatment with CuNPs (10, 15, 20, 25, and 30 µM) and Bleomycin (10 µg/mL) resulted in a significant elevation of ROS levels, surpassing those observed with Bleomycin alone (p<0.01), suggesting a synergistic enhancement of oxidative stress.

Assessment of Lipid Peroxidation

MDA levels, a marker of lipid peroxidation, were significantly elevated in MCF-7 cells treated with CuNPs in combination with Bleomycin. The co-administration of CuNPs (10, 15, 20, 25, and 30 µM) and Bleomycin (10 µg/mL) resulted in a notable increase in MDA levels compared to individual treatments , indicating greater oxidative damage to cellular membranes.

Reduction of Intracellular Glutathione (GSH) Levels

GSH depletion was observed following treatment with CuNPs and Bleomycin. CuNPs (10, 15, 20, 25, and 30 µM) combined with Bleomycin (10

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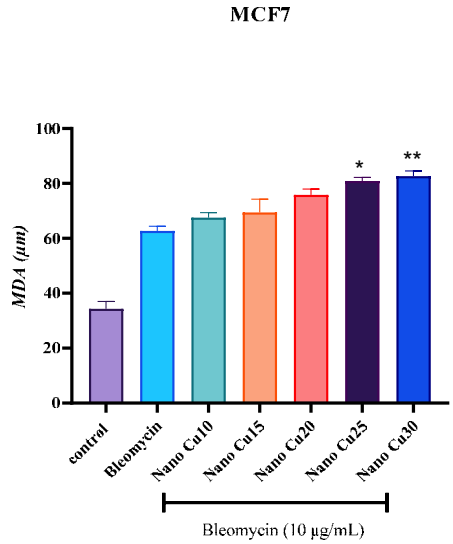

Fig. 4. MDA levels in MCF-7 breast cancer cells after 48-hour treatment with CuNPs at concentrations of 10, 15, 20, 25, and 30 µM, both alone and in combination with 10 µg/mL Bleomycin. The combination therapy significantly increases MDA levels compared to individual treatments (p<0.01), suggesting enhanced oxidative membrane damage. * (p<0.05) and ** (p<0.01) versus Bleomycin group.

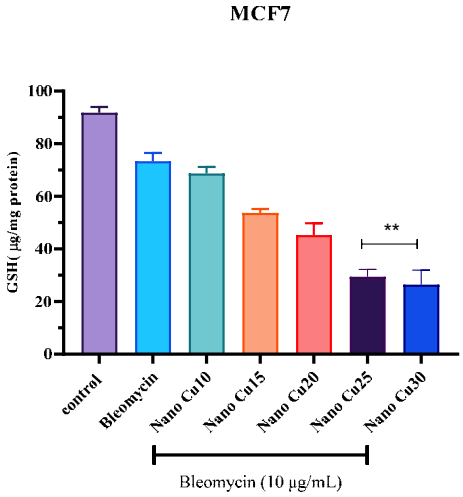

Fig. 5. GSH levels in MCF-7 breast cancer cells after 48-hour treatment with CuNPs at concentrations of 10, 15, 20, 25, and 30 µM, both alone and in combination with 10 µg/mL Bleomycin. The combination therapy significantly reduces GSH levels compared to individual treatments (p<0.01), highlighting the involvement of oxidative stress in cytotoxicity. ** (p<0.01) versus Bleomycin group.

µg/mL) significantly reduced GSH levels compared to individual treatments, emphasizing the role of oxidative stress in the cytotoxic mechanism.

Synergistic Cytotoxicity Mediated by Oxidative Stress

Overall, the findings demonstrate that CuNPs combined with Bleomycin exert a synergistic cytotoxic effect on MCF-7 breast cancer cells. This effect is likely mediated through enhanced oxidative stress, as evidenced by increased ROS production, lipid peroxidation, and depletion of intracellular GSH levels.

DISCUSSION

Breast cancer remains one of the most prevalent malignancies worldwide, necessitating the development of novel therapeutic strategies that enhance efficacy while minimizing adverse effects(37). Bleomycin is a widely used chemotherapeutic agent; however, its long-term clinical application is limited due to dose-dependent pulmonary toxicity and drug resistance(38). Recent

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advancements in nanotechnology have explored the potential of metal-based nanoparticles, including CuNPs, as adjuvant therapies to potentiate the cytotoxic effects of chemotherapeutic agents while mitigating their limitations(39). This study investigated the synergistic cytotoxicity of CuNPs in combination with Bleomycin in MCF-7 breast cancer cells, emphasizing oxidative stress-induced cell death mechanisms.

The MTT assay demonstrated a dose-dependent reduction in MCF-7 cell viability upon treatment with Bleomycin alone, as well as an enhanced cytotoxic effect when combined with CuNPs. This enhanced cytotoxicity suggests a potential interaction between CuNPs and Bleomycin, leading to increased cell death. Several mechanisms may account for this synergistic interaction, including the ability of CuNPs to disrupt redox homeostasis, enhance ROS production, and potentiate DNA damage, thereby amplifying Bleomycin's cytotoxic effects(40).

Oxidative stress plays a critical role in the cytotoxicity of both CuNPs and Bleomycin. The generation of ROS is one of the primary mechanisms by which CuNPs exert their toxic effects on cancer cells(41). Copper ions can undergo redox cycling via Fenton-like reactions, leading to excessive ROS production. These reactions result in the accumulation of hydroxyl radicals and superoxide anions, which contribute to oxidative damage in cellular components(42). Bleomycin, on the other hand, is known to induce ROS production via redox cycling of its quinone moiety(43). The combination of CuNPs and Bleomycin likely exacerbates ROS generation beyond the threshold of cellular antioxidant defenses, overwhelming the intrinsic antioxidant response and leading to oxidative damage-induced cell death(44, 45).

The increase in MDA levels in MCF-7 cells treated with CuNPs and Bleomycin suggests a significant degree of lipid peroxidation. Lipid peroxidation is a hallmark of oxidative stress-mediated cytotoxicity, in which free radicals attack polyunsaturated fatty acids within the plasma membrane, leading to the formation of lipid peroxides and aldehyde byproducts such as MDA(46, 47). The peroxidation of membrane lipids results in loss of membrane integrity, increased permeability, and subsequent cell death via necrosis or apoptosis(48). The observed increase in MDA levels in the combination treatment group strongly suggests that CuNPs enhance Bleomycin -induced oxidative membrane damage, thereby amplifying its cytotoxic effects.

Glutathione is a key antioxidant that plays a pivotal role in cellular defense against oxidative stress(49). The significant depletion of intracellular GSH levels in cells treated with CuNPs and Bleomycin indicates an overwhelmed antioxidant defense system. The depletion of GSH suggests that the increased ROS levels induced by the combination therapy may have exhausted the cellular antioxidant capacity, rendering the cells more susceptible to oxidative damage. Additionally, the oxidation of GSH to glutathione disulfide (GSSG) further contributes to redox imbalance and initiates apoptotic pathways through activation of pro-apoptotic proteins(50).

One of the major mechanisms of Bleomycin -induced cytotoxicity is its ability to intercalate into DNA and inhibit topoisomerase II, leading to DNA strand breaks and apoptosis(51). The presence of CuNPs may exacerbate DNA damage through ROS-induced oxidative stress, causing additional DNA fragmentation and activation of apoptosis. This combination therapy is likely to enhance the intrinsic (mitochondrial) apoptotic pathway, as excessive ROS levels disrupt mitochondrial integrity, leading to cytochrome c release(52). Cytochrome c subsequently interacts with apoptotic protease-activating factor 1 (Apaf-1), triggering the activation of caspase-9 and ultimately caspase-3, which executes the apoptotic program. Additionally, poly (ADP-ribose) polymerase (PARP) cleavage, a hallmark of apoptosis, further confirms the activation of cell death pathways(53, 54).

Moreover, the interplay between Bleomycin and CuNPs may contribute to p53-dependent apoptotic signaling. The excessive oxidative DNA damage caused by ROS can activate p53, leading to cell cycle arrest and programmed cell death(55). This suggests that CuNPs not only enhance the cytotoxic effects of Bleomycin but also reinforce apoptotic pathways through both mitochondrial and p53-mediated mechanisms. Given the central role of oxidative stress in this process, the extent of ROS production and the subsequent cellular antioxidant response will be critical determinants of the overall therapeutic efficacy and toxicity of this combination therapy.

Comparing these results with previous studies on metal-based nanoparticles in cancer therapy, similar trends have been observed with other

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nanoparticles, such as silver nanoparticles (AgNPs) and iron oxide nanoparticles (Fe3O4 NPs), which also exhibit ROS-mediated cytotoxicity(56, 57). For instance, AgNPs have been shown to induce mitochondrial dysfunction and oxidative DNA damage, leading to apoptosis in breast cancer cells(58). However, the advantage of CuNPs lies in their dual role as both a catalytic enhancer of ROS generation and a redox-active metal capable of interfering with essential cellular antioxidant systems, making them a particularly effective candidate for combination therapy(59). Moreover, unlike gold nanoparticles (AuNPs), which primarily serve as drug carriers with minimal intrinsic toxicity, CuNPs exhibit intrinsic cytotoxic effects, further strengthening their potential as a standalone or adjunct therapeutic agent(60).

A key mechanistic insight from this study is the role of CuNPs in exacerbating oxidative stress by interfering with antioxidant defense mechanisms, particularly by depleting intracellular GSH levels. The depletion of GSH, a critical intracellular antioxidant, makes cells more vulnerable to ROS-induced damage, leading to increased lipid peroxidation and subsequent membrane instability(61). Similar effects have been reported with other transition metal nanoparticles, such as ZnO NPs, which also induce oxidative stress-related apoptosis in various cancer cell lines(62). However, CuNPs exhibit unique redox cycling properties that contribute to their higher efficacy in disrupting redox balance, making them an attractive alternative to other metal-based nanotherapeutics(42).

The observed synergy between CuNPs and Bleomycin in this study suggests a potential strategy to overcome chemoresistance in breast cancer cells. Bleomycin, a widely used anthracycline chemotherapeutic, exerts its cytotoxic effects primarily through DNA intercalation and topoisomerase II inhibition, leading to apoptotic cell death. However, its clinical application is often limited by resistance mechanisms, including enhanced DNA repair, drug efflux, and increased antioxidant defenses(63). By introducing CuNPs into the treatment regimen, these resistance mechanisms can be mitigated through heightened oxidative stress, thereby sensitizing cancer cells to Bleomycin -induced cytotoxicity. Previous studies on metal nanoparticle-drug combinations have reported similar findings, with metal-based nanocarriers effectively overcoming multidrug resistance by disrupting cellular redox equilibrium and promoting apoptosis(64).

In contrast to studies utilizing non-metallic nanoparticles such as polymeric or lipid-based nanocarriers, which primarily function as drug delivery systems, CuNPs contribute to cancer therapy beyond passive drug transport(65). Their intrinsic cytotoxic properties, coupled with their ability to amplify chemotherapy-induced oxidative stress, set them apart from conventional nanocarriers(66). Additionally, while iron-based nanoparticles such as Fe3O4 have been explored for their potential in cancer therapy, their effects are often mediated through magnetic hyperthermia rather than direct cytotoxicity(67). In comparison, CuNPs provide a more direct and aggressive approach by actively generating ROS and disrupting cellular homeostasis(68).

Overall, this study provides strong evidence that CuNPs, when combined with Bleomycin, significantly enhance therapeutic efficacy through oxidative stress-mediated pathways. The findings suggest that CuNPs can be used to potentiate the effects of conventional chemotherapeutics, offering a promising strategy for improving treatment outcomes in breast cancer. Future studies should explore the long-term effects of CuNPs - Bleomycin combination therapy, particularly in in vivo models, to assess potential toxicity and systemic biodistribution. Additionally, investigating the molecular pathways involved in CuNPs mediated ROS generation and apoptosis induction could further elucidate their role as an emerging nanotherapeutic in oncology.

Potential Clinical Implications and Future Directions

The findings of this study suggest that CuNPs can significantly enhance the cytotoxic effects of Bleomycin in breast cancer cells through oxidative stress-mediated mechanisms. The observed increase in ROS generation, lipid peroxidation, and depletion of intracellular glutathione highlights the potential of CuNPs as an adjuvant in breast cancer therapy. However, before clinical translation, several crucial aspects need to be addressed. One major concern is the potential for off-target toxicity, as systemic administration of CuNPs may lead to damage in normal tissues. Future studies should focus on developing tumor-targeted CuNPs by functionalizing them with tumor-specific ligands to enhance selective accumulation in cancer cells. Additionally, while in vitro findings provide

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strong evidence of synergistic cytotoxicity, in vivo studies are essential to assess pharmacokinetics, biodistribution, and systemic toxicity. Investigating these parameters will help determine the therapeutic index and safety profile of CuNPs based combination therapy.

Moreover, optimization of the CuNPs to Bleomycin ratio is critical to achieving maximum therapeutic efficacy while minimizing potential adverse effects. Excessive ROS production could lead to unintended toxicity, necessitating precise dose adjustments for clinical application. Another important consideration is the stability and clearance pathways of CuNPs, as long-term retention and aggregation could pose toxicity risks. Therefore, understanding their biodegradation and elimination from the body is essential for clinical feasibility. Comparatively, other studies exploring metal-based nanoparticles, such as gold and silver nanoparticles, have demonstrated similar ROS-mediated cytotoxic effects, though CuNPs exhibit distinct redox properties that may provide a stronger synergistic interaction with Bleomycin. Further research into nanoparticle engineering, including coating strategies and controlled-release mechanisms, may enhance the therapeutic potential of CuNPs while mitigating toxicity concerns. This study lays the foundation for future investigations into CuNPs based combinational chemotherapy and underscores the necessity of translational research to bring this promising approach to clinical application.

CONCLUSION

This study provides compelling evidence that CuNPs enhance Bleomycin -induced cytotoxicity in MCF-7 breast cancer cells by increasing oxidative stress, inducing lipid peroxidation, depleting intracellular antioxidants, and promoting apoptotic cell death. These findings open new avenues for using CuNPs as a potential adjuvant to conventional chemotherapy, paving the way for novel combination therapies in breast cancer treatment. Future research should focus on optimizing nanoparticle formulations to maximize therapeutic benefits while minimizing adverse effects, ultimately advancing the clinical application of CuNPs based combination therapies.

CONFLICT OF INTERESTS

The author(s) declare that they have no competing interests.

REFERENCES

1. Nicoletto RE, Ofner CM 3rd. Cytotoxic mechanisms of doxorubicin at clinically relevant concentrations in breast cancer cells. Cancer Chemother Pharmacol. 2022;89(3):285-311. https://doi.org/10.1007/s00280-022-04400-y
2. Hadisaputri YE, Habibah U, Abdullah FF, Halimah E, Mutakin M, Megantara S, et al. Antiproliferation activity and apoptotic mechanism of soursop (Annona muricata L.) leaves extract and fractions on MCF7 breast cancer cells. Breast Cancer (Dove Med Press). 2021;13:447-57. https://doi.org/10.2147/BCTT.S317682
3. Jałoszyński P, Kujawski M, Czub-Świerczek M, Markowska J, Szyfter K. Bleomycin-induced DNA damage and its removal in lymphocytes of breast cancer patients studied by comet assay. Mutat Res DNA Repair. 1997;385(3):223-33. https://doi.org/10.1016/S0921-8777(97)00046-3
4. Gatignol A, Durand H, Tiraby G. Bleomycin resistance conferred by a drug-binding protein. FEBS Lett. 1988;230(1-2):171-5. https://doi.org/10.1016/0014-5793(88)80665-3
5. Atienza JM, Roth RB, Rosette C, Smylie KJ, Kammerer S, Rehbock J, et al. Suppression of RAD21 gene expression decreases cell growth and enhances cytotoxicity of etoposide and bleomycin in human breast cancer cells. Mol Cancer Ther. 2005;4(3):361-8. https://doi.org/10.1158/1535-7163.MCT-04-0241
6. Shakiba D, Shabestari AM, Mokhtari T, Goodarzi MK, Saeed S, Zinatbakhsh Z, et al. Nanoliposomes Meet Folic Acid: A Precision Delivery System for Bleomycin in Cancer Treatment. Asian Pac J Cancer Biol. 2024;9(4):561-8. https://doi.org/10.31557/apjcb.2024.9.4.561-568
7. Moradi M, Goodarzi N, Faramarzi A, Cheraghi H, Hashemian AH, Jalili C. Melatonin protects rats testes against bleomycin, etoposide, and cisplatin-induced toxicity via mitigating nitro-oxidative stress and apoptosis. Biomed Pharmacother. 2021;138:111481. https://doi.org/10.1016/j.biopha.2021.111481
8. Wu F, Ma Y, Wang J, Ou H, Dang H, Zheng Y, et al. Bleomycin A5 suppresses Drp1-mediated mitochondrial fission and induces apoptosis in human nasal polyp-derived fibroblasts. Int J Mol Med. 2021;47(1):346-60. https://doi.org/10.3892/ijmm.2020.4797
9. Guo F, Xu F, Li S, Zhang Y, Lv D, Zheng L, et al. Amifostine ameliorates bleomycin-induced murine pulmonary fibrosis via NAD+/SIRT1/AMPK pathway-mediated effects on mitochondrial function and cellular metabolism. Eur J Med Res. 2024;29(1):68. https://doi.org/10.1186/s40001-023-01623-4
10. Sim S, Wong NK. Nanotechnology and its use in imaging and drug delivery. Biomed Rep. 2021;14(5):42. https://doi.org/10.3892/br.2021.1418
11. Zadeh FA, Bokov DO, Salahdin OD, Abdelbasset WK, Jawad MA, Kadhim MM, et al. Cytotoxicity evaluation of environmentally friendly synthesis Copper/Zinc bimetallic nanoparticles on MCF-7 cancer cells. Rend Lincei Sci Fis Nat. 2022;33(2):441-7. https://doi.org/10.1007/s12210-022-01064-x
12. Dehnoee A, Kalbasi RJ, Zangeneh MM, Delnavazi MR, Zangeneh A. Characterization, anti-lung cancer activity, and cytotoxicity of bio-synthesized copper nanoparticles by Thymus fedtschenkoi leaf extract. J Clust Sci.

2024;35(3):863-74. https://doi.org/10.1007/s10876-023-02512-w
13. Ghassemi-Barghi N, Varshosaz J, Etebari M, Dehkordi AJ. Role of recombinant human erythropoietin loading chitosan-tripolyphosphate nanoparticles in busulfan-induced genotoxicity: Analysis of DNA fragmentation via comet assay in cultured HepG2 cells. Toxicol In Vitro. 2016;36:46-52. https://doi.org/10.1016/j.tiv.2016.07.001
14. Wang L, Jiang J, Ma J, Pang S, Zhang T. A review on advanced oxidation processes homogeneously initiated by copper (II). Chem Eng J. 2022;427:131721. https://doi.org/10.1016/j.cej.2021.131721
15. Ge EJ, Bush AI, Casini A, Cobine PA, Cross JR, DeNicola GM, et al. Connecting copper and cancer: from transition metal signalling to metalloplasia. Nat Rev Cancer. 2022;22(2):102-13. https://doi.org/10.1038/s41568-021-00417-2
16. Wang X, Zhou M, Liu Y, Si Z. Cope with copper: From copper linked mechanisms to copper-based clinical cancer therapies. Cancer Lett. 2023;561:216157. https://doi.org/10.1016/j.canlet.2023.216157
17. Huynh DTN, Jin Y, Myung CS, Heo KS. Ginsenoside Rh1 induces MCF-7 cell apoptosis and autophagic cell death through ROS-mediated Akt signaling. Cancers (Basel). 2021;13(8):1892. https://doi.org/10.3390/cancers13081892
18. Shokrzadeh M, Mortazavi P, Moghadami A, Khayambashi B, Motafeghi F. Synergistic antiproliferative and anticancer activity of carotenoid lutein or coenzyme Q10 in combination with doxorubicin on the MCF7 cell line. Appl In Vitro Toxicol. 2021;7(4):167-74. https://doi.org/10.1089/aivt.2021.0008
19. Motafeghi F, Shahsavari R, Mortazavi P, Babaei A, Mojaveri PS, Khojasteh OA, et al. Metformin and Aspirin: Anticancer effects on A549 and PC3 cancer cells and the mechanisms of action. Toxicol Res (Camb). 2023;12(4):702-8. https://doi.org/10.1093/toxres/tfad060
20. Machado KL, Marinello PC, Silva TNX, Silva CFN, Luiz RC, Cecchini R, et al. Oxidative stress in caffeine action on the proliferation and death of human breast cancer cells MCF-7 and MDA-MB-231. Nutr Cancer. 2021;73(8):1378-88. https://doi.org/10.1080/01635581.2020.1795693
21. Ashari S, Naghsh N, Salari Y, Barghi NG, Bagheri A. Dimethyl fumarate attenuates di-(2-ethylhexyl) phthalate-induced nephrotoxicity through the Nrf2/HO-1 and NF-κB signaling pathways. Inflammation. 2023;46(1):453-67. https://doi.org/10.1007/s10753-022-01746-6
22. Shirzadi-Ahodashti M, Mizwari ZM, Jafarkhani B, Abbastabar M, Motafeghi F, Lalerdi FS, et al. Biogenic synthesis of spherical-shaped noble metal nanoparticles using Vicia faba extract (X@VF, X=Au, Ag) for photocatalytic degradation of organic hazardous dye and their in vitro antifungal, antibacterial and anticancer activities. Inorg Chem Commun. 2022;146:110042. https://doi.org/10.1016/j.inoche.2022.110042
23. Wang X, Wang WX. Cu(I)/Cu(II) released by Cu nanoparticles revealed differential cellular toxicity related to mitochondrial dysfunction. Environ Sci Technol. 2023;57(26):9548-58. https://doi.org/10.1021/acs.est.3c00864
24. Long X, Liu M, Nan Y, Chen Q, Xiao Z, Xiang Y, et al. Revitalizing ancient mitochondria with nano-strategies: mitochondria-remedying nanodrugs concentrate on disease control. Adv Mater. 2024;36(18):2308239. https://doi.org/10.1002/adma.202308239
25. Ebrahimi R, Shokrzadeh M, Ghassemi Barghi N. Effects of melatonin on the Bisphenol-A-induced cytotoxicity and genetic toxicity in colon cancer cell lines, normal gingival cell lines, and bone marrow stem cell lines. Cancer Inform. 2021;20:11769351211056295. https://doi.org/10.1177/11769351211056295
26. Motafeghi F, Gerami M, Mortazavi P, Khayambashi B, Ghassemi-Barghi N, Shokrzadeh M. Green synthesis of silver nanoparticles, graphene, and silver-graphene nanocomposite using Melissa officinalis ethanolic extract: Anticancer effect on MCF-7 cell line. Iran J Basic Med Sci. 2023;26(1):57-66.
27. Motafeghi F, Fakhri B MS, Ghassemi Barghi N. Mechanisms of ARA290 in counteracting cadmium-triggered neurotoxicity in PC12 cells. Toxicol Res (Camb). 2025;14(1):tfaf023. https://doi.org/10.1093/toxres/tfaf023
28. Shokrzadeh M, Motafeghi FS, Lotfizadeh A, Ghorbani M, Haddadi Kohsar A. Cytotoxicity and mineralization activity of calcium silicate-based root canal sealers compared to conventional resin-based sealer in human gingival fibroblast cells. Int J Dent. 2023;2023:4376579. https://doi.org/10.1155/2023/4376579
29. Shokrzadeh M, Ghassemi-Barghi N. Antioxidant and genoprotective effects of amifostine against irinotecan toxicity in human hepatoma cells. Int J Canc Res Therap.
30. vi P, Shokrzadeh M. Anticancer effect of paroxetine and amitriptyline on HT29 and A549 cell lines. Toxicol In Vitro. 2023;87:105532. https://doi.org/10.1016/j.tiv.2022.105532
31. Ghassemi-Barghi N, Ehsanfar Z, Mohammadrezakhani O, Ashari S, Ghiabi S, Bayrami Z. Mechanistic approach for protective effect of ARA290, a specific ligand for the erythropoietin/CD131 heteroreceptor, against cisplatin-induced nephrotoxicity, the involvement of apoptosis and inflammation pathways. Inflammation. 2023;46(1):342-58. https://doi.org/10.1007/s10753-022-01737-7
32. Motafeghi F, Khayambashi B, Mortazavi P, Eghbali M, Salmanmahiny A, Shahsavari R, et al. Synergistic Effect of Selenium/Zinc with Sulfasalazine on the Human Colorectal Cancer Cell Line (HT-29). Appl In Vitro Toxicol. 2023;9(1):3-12. https://doi.org/10.1089/aivt.2022.0007
33. Motafeghi F, Mortazavi P, Shahsavari R, Shaker M, Mohammadi-Berenjestanaki H, Shokrzadeh M. Kombucha mushroom extract: anticancer, antioxidant, and antimicrobial properties. Appl In Vitro Toxicol. 2023;9(3):90-103. https://doi.org/10.1089/aivt.2023.0005
34. Motafeghi F, Mortazavi P, Shokrzadeh M. Anticancer activity of zinc oxide nanoparticles on prostate and colon cancer cell line. Toxicol Res (Camb). 2024;13(1):tfad127. https://doi.org/10.1093/toxres/tfad127
35. Shokrzadeh M, Mohammadpour A, Ghassemi-Barghi N, Hoseini V, Abediankenari S, Tabari YS. Metallothionein-2A (rs1610216&rs28366003) gene polymorphisms and the risk of stomach adenocarcinoma. Arq Gastroenterol. 2019;56(4):367-71. https://doi.org/10.1590/s0004-2803.201900000-69
36. Shokrzadeh M, Mohammadpour A, Modanloo M, Hassani M, Barghi NG, Niroomand P. Cytotoxic effects of aripiprazole on MKN45 and NIH3T3 cell lines and genotoxic effects on human peripheral blood lymphocytes. Arq Gastroenterol. 2019;56:155-9. https://doi.org/10.1590/s0004-2803.201900000-31
37. Trayes KP, Cokenakes SE. Breast cancer treatment. Am

(cc) BY

Fam Physician. 2021;104(2):171-8.
38. Yang W, Pan L, Cheng Y, Wu X, Huang S, Du J, et al. Amifostine attenuates bleomycin-induced pulmonary fibrosis in mice through inhibition of the PI3K/Akt/mTOR signaling pathway. Sci Rep. 2023;13(1):10485. https://doi.org/10.1038/s41598-023-34060-8
39. Kumar P, Mangla B, Javed S, Ahsan W, Musyuni P, Sivadasan D, et al. A review of nanomaterials from synthetic and natural molecules for prospective breast cancer nanotherapy. Front Pharmacol. 2023;14:1149554. https://doi.org/10.3389/fphar.2023.1149554
40. Manohar A, Vijayakanth V, Vattikuti SP, Kim KH. A mini-review on AFe2O4 (A=Zn, Mg, Mn, Co, Cu, and Ni) nanoparticles: Photocatalytic, magnetic hyperthermia and cytotoxicity study. Mater Chem Phys. 2022;286:126117. https://doi.org/10.1016/j.matchemphys.2022.126117
41. Gupta G, Cappellini F, Farcal L, Gornati R, Bernardini G, Fadeel B. Copper oxide nanoparticles trigger macrophage cell death with misfolding of Cu/Zn superoxide dismutase 1 (SOD1). Part Fibre Toxicol. 2022;19(1):33. https://doi.org/10.1186/s12989-022-00467-w
42. Ameh T, Gibb M, Stevens D, Pradhan SH, Braswell E, Sayes CM. Silver and copper nanoparticles induce oxidative stress in bacteria and mammalian cells. Nanomaterials (Basel). 2022;12(14):2402. https://doi.org/10.3390/nano12142402
43. Pan L, Cheng Y, Yang W, Wu X, Zhu H, Hu M, et al. Nintedanib ameliorates bleomycin-induced pulmonary fibrosis, inflammation, apoptosis, and oxidative stress by modulating PI3K/Akt/mTOR pathway in mice. Inflammation. 2023;46(4):1531-42. https://doi.org/10.1007/s10753-023-01825-2
44. Saddik MS, Elsayed MM, Abdel-Rheem AA, El-Mokhtar MA, Mosa ES, Al-Hakkani MF, et al. A Novel C@Fe@Cu nanocomposite loaded with doxorubicin tailored for the treatment of hepatocellular carcinoma. Pharmaceutics. 2022;14(9):1845. https://doi.org/10.3390/pharmaceutics14091845
45. Shokrzadeh M, Goleij P, Behravan E, Ghassemi-Barghi N, Salehabadi Y, Rezaei A. Association between diabetics and intestinal cancer with the risk of mutation in CD38 gene in Iranian population. Arq Gastroenterol. 2020;57(2):137-43. https://doi.org/10.1590/s0004-2803.202000000-25
46. Liao HH, Ding W, Zhang N, Zhou ZY, Ling Z, Li WJ, et al. Activation of AMPKα2 attenuated doxorubicin-induced cardiotoxicity via inhibiting lipid peroxidation associated ferroptosis. Free Radic Biol Med. 2023;205:275-90. https://doi.org/10.1016/j.freeradbiomed.2023.06.004
47. Ahadi H, Shokrzadeh M, Hosseini-khah Z, Ghassemi Barghi N, Ghasemian M, Emami S. Conversion of antibacterial quinolone drug levofloxacin to potent cytotoxic agents. J Biochem Mol Toxicol. 2023;37(6):e23334. https://doi.org/10.1002/jbt.23334
48. Akpinar D, Mercan T, Demir H, Ozdemir S, Demir C, Kavak S. Protective Effects of Thymoquinone on Doxorubicin-induced Lipid Peroxidation and Antioxidant Enzyme Levels in Rat Peripheral Tissues. Pak Vet J. 2023;43(4):[page range missing]. https://doi.org/10.21203/rs.3.rs-2702180/v1
49. Georgiou-Siafis SK, Tsiftsoglou AS. The key role of GSH in keeping the redox balance in mammalian cells: mechanisms and significance of GSH in detoxification via formation of conjugates. Antioxidants (Basel). 2023;12(11):1953. https://doi.org/10.3390/antiox12111953
50. Liu T, Sun L, Zhang Y, Wang Y, Zheng J. Imbalanced GSH/ROS and sequential cell death. J Biochem Mol Toxicol. 2022;36(1):e22942. https://doi.org/10.1002/jbt.22942
51. Jacinto MP, Fried SD, Greenberg MM. Intracellular Formation of a DNA Damage-Induced, Histone Post-Translational Modification Following Bleomycin Treatment. J Am Chem Soc. 2022;144(17):7600-5. https://doi.org/10.1021/jacs.2c02880
52. Syahputra RA, Harahap U, Harahap Y, Gani AP, Dalimunthe A, Ahmed A, et al. Vernonia amygdalina ethanol extract protects against doxorubicin-induced cardiotoxicity via TGFβ, cytochrome c, and apoptosis. Molecules. 2023;28(11):4305. https://doi.org/10.3390/molecules28114305
53. Chen JK, Ramesh S, Islam MN, Shibu MA, Kuo CH, Hsieh DJY, et al. Ohwia caudata inhibits doxorubicin-induced cardiotoxicity by regulating mitochondrial dynamics via the IGF-IIR/p-Drp1/PARP signaling pathway. Biotechnol Appl Biochem. 2024;71(5):1181-94. https://doi.org/10.1002/bab.2620
54. Bakhshoudeh M, Mehdizadeh K, Hosseinkhani S, Ataei F. Upregulation of apoptotic protease activating factor-1 expression correlates with anti-tumor effect of taxane drug. Med Oncol. 2021;38(8):88. https://doi.org/10.1007/s12032-021-01532-8
55. Hanson RL, Batchelor E. Coordination of MAPK and p53 dynamics in the cellular responses to DNA damage and oxidative stress. Mol Syst Biol. 2022;18(12):e11401. https://doi.org/10.15252/msb.202211401
56. Althomali A, Daghestani MH, Almukaynizi FB, Al-Zahrani SA, Awad MA, Merghani NM, et al. Anti-colon cancer activities of green-synthesized Moringa oleifera-AgNPs against human colon cancer cells. Green Process Synth. 2022;11(1):545-54. https://doi.org/10.1515/gps-2022-0052
57. Wang Y, Liu X, Ma S, He X, Guo C, Liang Z, et al. Progress in cancer therapy with functionalized Fe3O4 nanomaterials. Front Mater Sci. 2023;17(3):230658. https://doi.org/10.1007/s11706-023-0658-4
58. Danışman-Kalındemirtaş F, Kariper İA, Üstündağ H, Özsoy C, Erdem-Kuruca S. Antiproliferative effects of 5FU-AgNPs on different breast cancer cells. J Taibah Univ Sci. 2024;18(1):2354573. https://doi.org/10.1080/16583655.2024.2354573
59 Cui L, Gouw AM, LaGory EL, Guo S, Attarwala N, Tang Y, et al. Mitochondrial copper depletion suppresses triple-negative breast cancer in mice. Nat Biotechnol. 2021;39(3):357-67. https://doi.org/10.1038/s41587-020-0707-9
60. Liu H, Shi W, Luo Y, Cui G, Kong X, Han L, et al. Green supported of Au nanoparticles over reduced graphene oxide: Investigation of its cytotoxicity, antioxidant and anti-human breast cancer properties. Inorg Chem Commun. 2021;134:108918. https://doi.org/10.1016/j.inoche.2021.108918
61. Zou L, Cheng G, Xu C, Liu H, Wang Y, Li N, et al. Copper nanoparticles induce oxidative stress via the heme oxygenase 1 signaling pathway in vitro studies. Int J Nanomedicine. 2021;16:1565-73. https://doi.org/10.2147/IJN.S292319
62. Aazami MA, Rasouli F, Ebrahimzadeh A. Oxidative damage, antioxidant mechanism and gene expression in tomato responding to salinity stress under in vitro conditions and application of iron and zinc oxide

nanoparticles on callus induction and plant regeneration. BMC Plant Biol. 2021;21(1):597. https://doi.org/10.1186/s12870-021-03379-7

63. Abou El-Reash YG, Al-Farraj ES, Adam FA, El-Moneim A, Abu El-Reash GM. Bleomycin-dependent DNA damage, erythrocyte hemolysis, antitumor MTT assay, and antimicrobial activity studies for Cd (II), Mn (II), Zn (II), Cr (III), and Fe (III) complexes of a multidentate carbohydrazone ligand. Appl Organomet Chem. 2024;38(7):e7539. https://doi.org/10.1002/aoc.7539
64. Xu JJ, Zhang WC, Guo YW, Chen XY, Zhang YN. Metal nanoparticles as a promising technology in targeted cancer treatment. Drug Deliv. 2022;29(1):664-78. https://doi.org/10.1080/10717544.2022.2039804
65. Kolahalam LA, Prasad K, Krishna PM, Supraja N, Shanmugan S. The exploration of bio-inspired copper oxide nanoparticles: synthesis, characterization and in-vitro biological investigations. Heliyon. 2022;8(6):[eID missing]. https://doi.org/10.1016/j.heliyon.2022.e09726
66. Pan Q, Peng X, Cun JE, Li J, Pu Y, He B. In-situ drug generation and controllable loading: rational design of copper-based nanosystems for chemo-photothermal cancer therapy. Chem Eng J. 2021;409:128222. https://doi.org/10.1016/j.cej.2020.128222
67. Karimi S, Namazi H. Fe3O4@PEG-coated dendrimer modified graphene oxide nanocomposite as a pH-sensitive drug carrier for targeted delivery of doxorubicin. J Alloys Compd. 2021;879:160426. https://doi.org/10.1016/j.jallcom.2021.160426
68. Chen X, Cai Q, Liang R, Zhang D, Liu X, Zhang M, et al. Copper homeostasis and copper-induced cell death in the pathogenesis of cardiovascular disease and therapeutic strategies. Cell Death Dis. 2023;14(2):105. https://doi.org/10.1038/s41419-023-05639-w

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