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Original Article

مقایسه اثرات نانوذرات نقره، مس و اکسید روی بر بیان ژن فاکتور نکروز تومور α (TNF-α) در موش Balb/C آلوده به *شریشیا کلی*

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چکیده

سابقه و هدف: تعداد افراد مبتلا به باکتری‌های بیماری‌زا در حال افزایش است. آنتی‌بیوتیک‌های رایج به دلیل سازوکارهای ایجاد مقاومت، کارایی خود را از دست می‌دهند. فناوری نانو رویکردی به نام عوامل ضدمیکروبی مبتنی بر نانوذرات معرفی کرده است که قادر به مهار رشد باکتری‌ها می‌باشد.

مواد و روش‌ها: حداقل غلظت مهارکنندگی (MIC) نانوذرات نقره (Ag)، اکسید روی (ZnO) و مس (Cu) علیه *Escherichia coli* ATCC 25922 با استفاده از روش رقت‌سازی در کشت مایع تعیین شد. ٥٤ موش نر نژاد Balb/C به ٩ گروه تقسیم شدند، به‌صورت داخل صفاقی با *E.coli* (CFU/mL 10^8) آلوده شده و با نانوذرات ZnO، Ag و Cu (در دز MIC) یا ایمی‌پنم (گروه کنترل) تحت درمان قرار گرفتند. ٢٤ ساعت پس از درمان، RNA طحال استخراج شد و بیان ژن فاکتور نکروز تومور آلفا (TNF-α) با استفاده از Real-time PCR سنجیده شد.

یافته‌ها: MIC نانوذرات Ag برابر ١٢٥ میکروگرم بر میلی‌لیتر و برای ZnO و Cu برابر ٦٢/٥ میکروگرم بر میلی‌لیتر بود که اثربخشی بهتر نانوذرات ZnO و Cu را نشان داد. بیان ژن TNF-α در گروه‌های دریافت‌کننده ایمی‌پنم و نانوذرات نقره به‌طور معنی‌داری کمتر از گروه‌های دریافت‌کننده ZnO و Cu بود ($p < 0.05$).

نتیجه‌گیری: صرف‌نظر از آنتی‌بیوتیک‌ها، نانوذرات نیز خاصیت ضدمیکروبی علیه باکتری‌هایی نظیر *E. coli* دارند. این ویژگی‌ها آن‌ها را به گزینه‌ای مناسب برای درمان بیماری‌های عفونی تبدیل می‌کند.

واژگان کلیدی: *Escherichia coli*، نانوذرات، نقره، اکسید روی، مس، فاکتور نکروز توموری آلفا.

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Original Article

Comparison effects of silver, copper, and zinc oxide nanoparticles on the tumor necrosis factor-α (TNF-α) gene expression in infected Balb/C mice with *Escherichia coli*

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Abstract

Background & Objectives: The number of people infected by pathogenic bacteria is increasing. Routine antibiotics lose their effectiveness due to the strategies of these bacteria in resistant to antibiotics. Nanotechnology has introduced an approach called nanoparticle-based antimicrobial agents to inhibit bacteria growth.

Materials & Method: Minimum inhibitory concentrations (MICs) of silver (Ag), zinc oxide (ZnO), and copper (Cu) NPs against *Escherichia coli* ATCC 25922 were determined using microbroth dilution. Balb/C male mice (n=54) were divided into nine groups, infected intraperitoneally with E. coli (10^8 CFU/mL), and treated with Ag, ZnO, or Cu NPs (MIC doses) or imipenem (control). Spleen tissue RNA was extracted 24 h post-treatment, and tumor necrosis factor-α (TNF-α) gene expression was quantified via Real-time PCR.

Results: MICs were 125 µg/mL (Ag) and 62.5 µg/mL (ZnO, Cu), indicating superior efficacy of ZnO and Cu NPs. TNF-α expression was significantly lower in imipenem and Ag NP groups compared to ZnO and Cu NP groups ($p < 0.05$).

Conclusion: Regardless of the antibiotics, NPs also exhibit an antimicrobial property against bacteria like *E. coli*. These characteristics make them the proper candidate for the treatment of infectious diseases.

Keywords: *Escherichia coli,* Nanoparticles, Silver, Zinc oxide, Copper, Tumor necrosis factor-alpha.

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Introduction

Over the past decade, antibiotic resistance has been one of the human concerns because many infectious diseases originating from bacterial pathogens are being increased dramatically, and involving microbial agents reveals more resistance toward conventional antibiotics. *E. coli* is the second most common known bacterial infection in the community (1). *E. coli* is the second most common known bacterial infection in the community (2).According to the Centers for Disease Control and Prevention (CDCP), infections

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derived from *Escherichia coli* O157:H7 increased from 73,480 to 265,000 cases between the years 20052017 in the United States (3). Regardless of antibiotic resistance, the microflora in the human gut is affected by the irregular intake of antibiotics. For example, many beneficial bacteria such as probiotics are eliminated that play a significant role in digestion (4), control of sugar and cholesterol, and improving the immune system against pathogens (5). The design and production of novel and effective antibiotics is a complicated and time-consuming process, and it seems that the pathogens have found strategies to counteract the effects of the available antibiotics (6). Fortunately, scientists are recruiting innovative methods to overcome pathogens producing infections in humans and animals. Currently, nanotechnology has revolutionized many sciences like biomedicine, and the emergence of nanoparticle-based antimicrobial agents. (7). Thanks to the action of nanotechnology in nano dimensions, the efficiency of the nanomaterials in killing or reducing the microbes, and drug delivery are high and acceptable (8). Virulence factors of the pathogens can suppress the phagocytic immune response when the body is engaged in the infection (9). By forming biofilms on surfaces, bacteria can evade eradication by inhibiting phagocytosis by immune cells. (5). Treatment with nanoparticle-based antimicrobial agents often is not able to recover immune system suppression ability, and that is why NPs do not reveal the maximum antimicrobial *in vivo* (10). Silver, copper, gold, and zinc are three significant elements with antimicrobial properties, which thousands of years ago, ancient people recognized this feature.The binding of nanoparticles to cells induces endocytosis, which may help protect against bacterial infection by disrupting pathogen adhesion or cellular invasion Some modified carriers have been designed with the ability to cross biological membranes at the nano level, to improve the effectiveness of drugs in the target (11). Today, silver nanoparticles (Ag NPs) (12), gold nanoparticles (Au NPs) (13), zinc oxide nanoparticles (ZnO NPs) (14), and copper nanoparticles (Cu NPs) (15), with a diameter of less than 100 nm, are prevalent NPs being used as antimicrobial agents. Owing to the unique physical structure (16), increased surface-to-volume ratio of a given mass of the NPs (NPs sizes) (8), and good interaction and penetration into the bacteria cell wall (17), the bactericidal property of the NPs is appropriate. Immediate cell damage by destroying the integrity and continuity of the cell membrane, oxidative stress, and apoptosis in the treated cells are the main mechanisms of the NPs (18). Besides, NPs prevent cell survival by increasing lactate dehydrogenase leakage, activating reactive oxygen species, modulating various signaling molecules, and DNA damage. Moreover, NPs prevent cell survival by increasing lactate dehydrogenase leakage (19), activating reactive oxygen species (20), modulating various signaling molecules (21), and DNA damage (21). Many studies have reported some NPs like silver classes, release Ag^{+} ions and are attached to the thiol groups in Gram-negative and Gram-positive bacterial proteins leading to disturbance in the duplication of DNA (22,23). Cytokines are proteins secreted by innate and adaptive immune cells that mediate many actions of these cells. Cytokines produced in response to antigens and other microorganisms like tumor necrosis factor-α (*TNF-α*) and interleukins (IL-1β, IL-6, and IL-10) are involved in inflammation and immunity (24).

TNF-α is one of the inflammatory cytokines and plays an affecting role in host defense, inflammation, and immune system function, which is related to the pathogenesis, development, and progression of various infections, autoimmune diseases, and malignant diseases (25). Many studies worked on models to understand the role of different NPs in increasing the release of pre and pro-inflammatory cytokines during infection (26-30), and all argued that the NPs boost the immune system separately or synergistically with neutrophils (31).

This study aimed to investigate the gene expression change associated with *TNF-α* cytokine release in mice infected with *E. coli* ATCC 25922 treated with Ag, Cu, and ZnO NPs. The novel aspect of the study is working with Balb/C mice that previously no researcher studied affecting the role of the imipenem antibiotic and NPs in *TNF-α* cytokine release in animal models. Besides, the minimum inhibitory concentrations (MICs) of three NPs against *E. coli* were investigated.

Materials and methods

A) Preparation and activation of microbial strain: In the first step, lyophilized powder of *E. coli* ATCC 25922, was transferred to Mueller Hinton Agar (MHA) medium and incubated for 24 h at 37 °C. To prepare bacterial suspension, it was essential to activate the microbial strain. For this purpose, one or two colonies of the 24h-culture of the *E. coli* were added to a tube containing sterile physiological serum and then shook to obtain an opaque medium containing bacteria (32). Obtained opacity was checked with a previously prepared control tube containing standard 0.5 McFarland to determine negative or false results (In terms of sensitivity or resistance) if the number of bacteria used is not appropriate. For more certainty, its absorbance at 640 nm was measured by spectrophotometer (Thermo Fisher Scientific, USA; Jenway 7315, UK), which is within the standard absorbance range of the studied microorganism.

B) Determination of the MICs of Ag, Cu, and ZnO NPs against E. coli: This test was performed by the microdilution method in a 96-wells microplate. Eight concentrations of each NPs were examined to obtain the MICs of NPs against *E. coli*, including concentrations of 0, 15.6, 31.25, 62.5, 125, 250, 500, and 1000 µg/ml). First, stocks with a concentration of 2000 µg/ml of NPs (Ag, Cu, and ZnO) were prepared, and in each of the wells, we added 95 µl of Mueller Hinton Broth (MHB) medium. Then, 100 µl of the stocks with a concentration of 2000 µg/ml was added to the first well of each of the two rows assigned to one nanoparticle. The NPs concentrations were obtained at 1000 µg/ml in the first well, and 100 µl from the first well was taken and added to the second well. This action continued for each NP in the corresponding rows until the last column. After mixing the medium with the target NPs, 100 µl from the last well was discarded. Then, 5 µl of the 24h-culture of the *E. coli* suspension (0.5 McFarland) was added to each well. The wells of the last two rows in each microplate were allocated to the negative and positive control containing the imipenem antibiotic. Next, the microplates were incubated for 24 h at 37 °C in a shaker incubator, and control samples were checked to confirm the accuracy of the tests. The MICs of the NPs and imipenem against *E. coli* were calculated based on the highest to the lowest concentration of the wells. Opaque wells indicate the growth of microorganisms. The test was repeated, and its validation was run based on the

instruction (33).

C) Antimicrobial effect of Ag, Cu, and ZnO NPs on E. coli in the animal model: In this study, 63 males BALB/c mice were purchased from Royan Research Institute in Tehran, and the mice were transferred to the animal room of the biology research unit located in Islamic Azad University, Central Tehran Branch, and were kept for 2-4 days to adapt to the new laboratory conditions. This study was approved by the Ethics Committee of the Islamic Azad University-Varamin (IR.IAU.VARAMIN.REC.1402.007), Tehran, Iran.

Mice were divided into nine groups, including seven mice in each group, group 1: healthy control of mice; groups 2, 3, and 4: Ag, Cu, and ZnO NPs controls; group 5: studied *E. coli*; groups 6, 7, and 8: mice infected with *E. coli* and treated with mentioned NPs; and group 9: mice infected with *E. coli* and treated with imipenem antibiotic. For the preparation of the animal model, mice in groups 1, 2, 3, and 4 were injected with only 100 Landa physiological serum by intraperitoneal injection, and groups 5, 6, 7, 8, and 9 were infected with Landa of *E. coli*, on the first day of the injection. About 3-4 h after injection, mice of groups 2, 3, 4, 6, 7, and 8 received intraperitoneally 100 Landa NPs (Minimum inhibitory concentrations). Mice of group 9 were treated with 100 Landa of imipenem according to the average weight of the mice, and other groups received physiological serum. The next day, three mice in each group were chosen randomly, as their spleens were separated, and RNAs were extracted according to the protocol. Other mice in each group were treated according to the first-day protocol, as this action was continued for five days (34).

D) RNA extraction and preparation of cDNA: For the preparation of RNA from spleen tissue cells, we cut a piece of the tissue and mixed it with the triazole. Extracts were obtained according to the CinnaPure-RNA extraction PR891620 kit (CinnaGen Co, Iran). Finally, the vials containing RNAs were stored at -80 °C (35). A cDNA synthesis kit was used (CinnaGen Co, Iran) to obtain cDNA from the extracted RNAs according to the following protocol. For this purpose, a total volume of 10 µl was supplied, including; 1 µl DNAse enzyme; 1 µl buffer; 8 µl deionized water; and 1 µg RNA template, and incubation was done at 37 °C for 30 min. To inactivate the DNAse enzyme, 1 µl EDTA at 65 °C for 10 min was used and then transferred to a plate of ice. The following materials were added to each microtube, including 2 µl oligo dT, 2 µl dNTP (10mM), 1 µl RT enzyme, 1.5 µl MgCl2, 4 µl 5X PrimeScript buffer, and 0.5 µl RNasin (40 Unit/µl). PCR reaction tubes were inserted at 37 °C for 60 min in a thermocycler. Then, samples remained in the thermocycler at 70 °C for 10 min to inactivate the RT enzyme, and finally, the reaction product was kept at -20 °C (36). The quality and homogenization of the cDNA samples were evaluated by the NanoDrop One C device (Thermo Scientific Co, US) (37).

E) Expression changes of TNF-α gene using Real-Time PCR: In this research, the SYBR green method (For binding to DNA), primers of *β-actin* and *TNF-α* genes, as well as cDNA were obtained from RNA extracted from cells infected with *E. coli*-NPs culture extract was applied in two separate reactions, and duplicated. Despite being priceless, sensitive, and effortless use of SYBR green, due to binding to two strands such as primer dimer and other non-specific bands, results are estimated to be higher than the original

concentration. Thereby, melting curve analysis was used to minimize the presence of non-specific products. To investigate the decrease or increase in the expression of the studied genes, we compared expression change with the internal control genes, in this study, β-actin was used as an internal control gene. The internal gene sequence was obtained from the NCBI website, and then specific primers were designed by the Primer Express Software v3.0.1. Finally, the designed primers were blasted by NCBI and Gene Runner Software to check the accuracy and specificity. The reverse and forward primers sequences used in this study are summarized in the following Table 1. PCR reaction was performed in a total volume of 25µl in microtube for each gene including, 1 µl cDNA; 1 µl forward primer; 1 µl reverse primer; 12.5 µl 2X SYBR green master mix; and 9.5 µl DEPC water. Cycles conditions of the reaction were as follows; One cycle at 95 ° C for 5 min as the first step for denaturation, 40 cycles at 95 °C for 20 sec, 40 cycles at 52 ° C for 15 sec, 40 cycles at 72 °C for 20 sec, and finally 55 °C to 94°C every 1 °C for 1 sec (38).

F) Statistical analysis of data: The statistical analysis of this study was calculated using SPSS 16 software, and the results were analyzed with one way ANOVA. The difference in target gene expression between the control and treated samples was calculated with Graphpad prism 6 and SPSS 16 software, method of Tukey's HSD post-hoc test ($P<0.05$). Real-time PCR data analysis was carried out based on threshold cycle comparison. The difference in the threshold cycles was obtained through cells treated with agents (NPs and antibiotic) and untreated cells. Using the specific formula (ΔΔCt), the ratio of the target gene to the reference gene of *β-actin* was calculated through 2-ΔΔCt. After performing the reaction and obtaining Ct using Rest 2009 software, we calculated ΔΔCt of each sample.

ΔCt = Ct target-Ct reference

ΔΔCt = ΔCt test sample - ΔCt control sample

Relative expression = 2 –ΔΔCt

Results

A) MICs of the NPs against E. coli: The minimum inhibitory concentrations (MICs) of the three tested nanoparticles (Ag, Cu, and ZnO) against *Escherichia coli* are summarized in Table 1. These concentrations were assessed using eight serial dilutions for each nanoparticle. The results indicated that as the concentration of nanoparticles increased, the bacterial growth decreased, suggesting a dose-dependent inhibitory effect. According to the broth microdilution method, the MIC was defined as the lowest concentration that prevented visible bacterial growth (i.e., no turbidity in the well).

The MICs for Cu and ZnO nanoparticles were both 62.5 µg/mL, while the MIC for Ag nanoparticles was 125 µg/mL. This finding indicates that Cu and ZnO NPs have greater antibacterial potency against *E. coli* compared to Ag NPs, as they inhibited bacterial growth at lower concentrations.

B) Expression changes of TNF-α gene in different treatment groups: To evaluate the host immune response to the different treatments, *TNF-α* gene expression was measured in the spleen tissues of infected mice using Real-time PCR. The specific sequences of primers used for TNF-α and β-actin genes are presented in Table 1. The *β- actin* gene served as the internal control.

Following one-day treatment with NPs and imipenem, the *TNF-α* expression levels significantly increased in all treated groups compared to the infected control group. The

most pronounced expression was observed in the imipenem-treated group ($P < 0.001$), followed by Ag ($P < 0.001$), Cu ($P < 0.005$), and ZnO ($P < 0.05$) nanoparticle groups, indicating a significant inflammatory response induced by these treatments (Figure 4).

Table 1: Sequence of primers used in Real-time PCR.

Gene	Primer sequence
TNF-α	Forward: 5'- CCAGGAGAAAGTCAGCCTCCT -3¢ Revers: 5'- TCATACCAGGGCTTGAGCTCA -3'
B-actin	Forward: 5'- AGAGCTATGAGCTGCCTGACG -3' Revers: 5'- CTGCATCCGGTCAGCGATAC -3'

After five days of treatment, similar trends were observed (Figure 2), where imipenem again led to the highest *TNF-α* expression ($P < 0.001$), followed by Ag NPs ($P < 0.001$), Cu NPs ($P = 0.002$), and ZnO NPs ($P = 0.1164$). It is notable that although ZnO NPs caused an increase in expression, the difference was not statistically significant after five days.

Comparing the expression levels over time, the fold change in *TNF-α* expression was higher at day five than day one in all treatment groups, suggesting that prolonged exposure to both antibiotics and nanoparticles may induce a stronger inflammatory response.

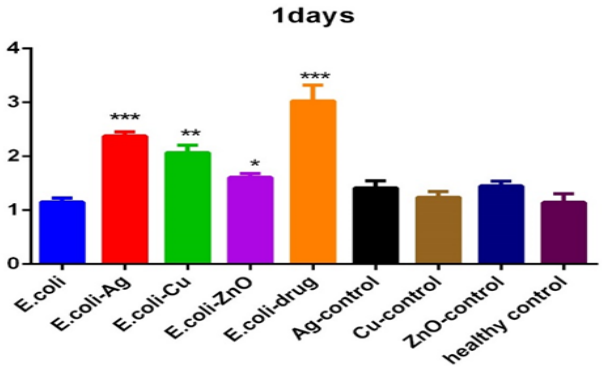

Figure 1: Change of *TNF-α* gene expression in the examined groups after one day of treatment. (*p < 0.05, **p < 0.01, ***p < 0.001).

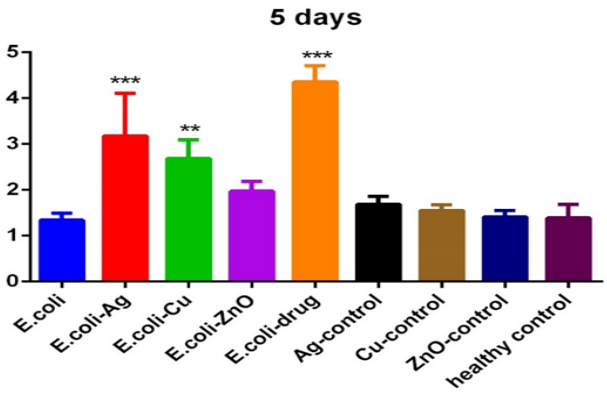

Figure 2: Change of *TNF-α* gene expression in the examined groups after five days of treatment. (*p < 0.05, **p < 0.01, ***p < 0.001).

Discussion

Today, the use of a wide range of antibiotics have been increased in the world, due to population growth and infection caused by bacterial pathogens. Unnecessary taking the antibiotics has brought many difficulties like antibiotic resistance and the appearance of disorders owing to disturbance of the microbial balance. Bacteria always have found strategies to escape antibacterial agents and transferred these resistance genes by vectors or plasmids [39]. Nanotechnology has provided many approaches to deal with antibiotic resistance, like nanoparticle-based antimicrobial agents. These agents penetrate the bacterial cell, interact with sulfur in membrane proteins, cleavage the DNA strand, and disturbance in transcription and translation which can result in bacterial death [40]. Antimicrobial properties of the NPs and their MICs against bacteria depend on many factors such as being Gram-positive or Gram-negative (Type of bacterium) [41], the applied dose and sustainability of NP [42], inherent power of the NP [43], environmental conditions like pH [8], and types of solvents used as a chemical reducer [44]. Often, Gram-positive bacteria like

Staphylococcus. Aureus exhibit more susceptibility to NPs compared with Gram-negative bacteria like *E. coli* due to the lack of an outer membrane containing lipopolysaccharide (LPS), cell physiology, and metabolism, Selahattin et al. emphasized [45]. In this study, we examined eight concentrations of Ag, Cu, and ZnO NPs in the *E. coli* (ATCC 25922) strain to obtain the MICs regarding each NP. Respectively, The MICs of Ag, Cu, and ZnO NPs were 125 µg/ml, 62.5 µg/ml, and 62.5 µg/ml. Although our results are associated with just Gram-negative bacterium, however, it does not deny the findings gained by other researchers. In a research conducted by Emami -Karvani and Chehrazi, they reported ZnO NPs with the MICs of 625 µg/ml and 78 µg/ml can inhibit *E. coli* and *S. aureus* growth, respectively [46]. This report confirms the findings of Reddy et al. (2007) who highlighted that Gram-negative bacteria have more resistance to NPs than Gram-negative bacteria, based on the obtained MICs [47]. In 2017, Li et al. [48] argued that the Ag NPs were able to stop the growth of *E.coli, S. aureus*, *S. epidermidis*, and *Pseudomonas aeruginosa* at the concentration of 0.5, 1, and 2 µg/ml, and environmental conditions such as pH and temperature do not change this value. Ag NPs play an important role in forming free radicals like reactive oxygen species (ROS), which cause damage to the bacterial cell membrane [49]. ROS species are highly reactive chemicals derived from O_2 such as peroxides and hydroxyl radicals that can increase oxidative stress in cells, and damage proteins, DNA, and intracellular systems such as the respiratory system [50]. Sharma and Chudasama evaluated the antibacterial activity of colloidal Cu NPs (Synthesized by chemical reduction method) against Gram-negative bacteria of *E.coli* and *Proteus vulgaris* under culture conditions [51]. MBC and MIC tests indicated dose-dependence bactericidal action and confirmed the antimicrobial activity of Cu NPs as a promising candidate has been reported previously by other studies [52-54]. Growth curves of *P. vulgaris* exhibited slower growth inhibition along with lower cytoplasmic leakage than *E.coli* due to increased membrane permeability of *E. coli* when interactions between Cu NPs and microorganism induces oxidative stress generated by ROS [51]. Another study carried out by Singh et al. (2021) highlighted the antimicrobial property of Ag NPs produced by the putative *Cedecea* sp. strain isolated from soil against two pathogenic bacteria with MICs of 12.5 and 6.25 µg/µl against *E. coli* and *P. aeruginosa*, respectively [55]. Our research is in agreement with mentioned studies that report NPs can inhibit bacteria growth like *E. coli* however, the value of the MIC varies based on the NPs sustainability, amount of inoculated microbe sample, rate of bacterial growth, purity, and age of microbial culture, as well as environmental and incubation circumstances [56].

Measuring specific cytokine levels like *TNF-α*, in infected human and animal cells treated with NPs is highly significant owing to predicting the effects of nanomaterials and the possibility of toxicity caused by inflammation as well as their role in cancer immunotherapy, assessment of cellular immunity, and drug design [57]. Many research emphasized that NPs cause the release of cytokines along with the inflammatory response in various cell lines through oxidative stress like the release of cytokines by titanium dioxide (TiO_2) NPs in a bronchial epithelial cell line [58] and glial cells [59], as well as cerium oxide (CeO_2 NPs) and ZnO in BEAS-2B cell lines [60]. ZnO NPs increase the

expression of interferon-gamma, *TNF-α*, and IL-12 in human immune cells [61]. It has also been reported that platinum (Pt), TiO_2, and diamond NPs cause the production of pro-inflammatory cytokines, dendritic cell maturation, and T cell activation and proliferation [62]. Beyerle et al. argued that crystalline silica oxide (SiO_2) NPs create the active species of the active agent and activate the *TNF-α* receptors, which produce the cellular transcription factor NF-κB and initiate the response [63]. The toxicity of NPs and their effects on the increase of inflammatory cytokines such as *TNF-α* due to oxidative stress are crucial issues, and more destructive effects of these NPs have been seen in high doses compared to lower doses. NPs may also generate large amounts of ROS that can induce the release of proinflammatory cytokines through NF-κB activation [64]. The results of this study show the significant effect of Ag, Cu, and ZnO NPs, respectively on *TNF-α* gene expression and the inflammatory response in mice compared with the control samples. Based on this, the importance of NPs as a replacement for antibiotics against *E. coli* is tangible. To compare NPs and antibiotic's strengths in *TNF-α* release, we evaluated the impact of imipenem antibiotics on *TNF-α* gene expression. Interestingly, after 1 and 5 days, the fold changes associated with imipenem were higher than studied NPs in mice after infection with *E. coli*. This result confirmed the findings of the earlier research by Toti et al. [65] and Saha et al. [66] reported antibiotics conjugated with NPs increased cytokine gene expression compared with only NPs. In a study by Tang et al. Ag NPs (400 mg/kg of mouse weight) induced the release of several cytokines, including IL-2 and MIP-1, and *TNF -α* levels in mice under treatment [67]. Jeong et al. reported that ZnO NPs induced *TNF-α* expression via the ROS-ERK-Egr-1 pathway in human keratinocytes and HaCaT cells [68]. The current study verifies mentioned research and other studies that investigated the influence of Ag NPs, Cu NPs, and ZnO NPs in the stimulation of cytokines release like *TNF-α* and increasing the immune systems [68-71]. Therefore, these NPs can be recruited as antimicrobial agents alone or create a synergistic correlation with conventional antibiotics to boost their efficiency in killing or stopping bacteria growth and inducing cytokine release.

Conclusion

The present study investigated the MICs of Ag, Cu, and ZnO NPs against *E. coli* (ATCC 25922) and showed that compared with other NPs, the Ag NPs in higher concentrations inhibit bacterial growth. Besides, among the different treatment groups, antimicrobial activity and inducing the *TNF-α* in the spleen tissue of mice infected with *E. coli* are significantly higher than NPs. These findings bring achievements to remedy or diagnose inflammatory diseases due to infections. NPs can be applied as medicine in the pharmaceutical industry when more studies work on different doses and tissues in the human model. This study suggests investigating the impact of a wide range of NPs in the stimulation of *TNF-α* through its gene expression change in other antibiotics-resistance bacteria, like food-borne pathogens.

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Conflict of Interest

The authors declare no conflict of interest. None of the authors have financial or personal relationships with organizations that could inappropriately influence the design, conduct, or reporting of this study.

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