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Zinc Oxide Nanoparticles Boosted Cefquinome Antimicrobial Efficacy and Ameliorated Its Side Effects on Buffalo Mammary Gland Epithelial Cells

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Treatment of mastitis induced by Staphylococcus aureus is notoriously difficult since *S. aureus* may rapidly develop biofilms and resistance to most antibiotics, including cefquinome (Cef). This study aimed to investigate whether zinc oxide nanoparticles (ZnONPs) would ameliorate cefquinome (Cef) side effects on buffalo mammary gland epithelial (MGE) cells and increase its antibacterial potential. We first evaluated the antibacterial impact of ZnONPs and/or Cef and found significantly higher antibacterial potential for the combined therapy against *Staphylococcus aureus* isolated from buffalo mastitic milk than individual therapy. Next, we used the MTT assay to evaluate the cytotoxic effects of ZnONPs and/or Cef on MGE cells and found significantly lowered cytotoxic effects for the combined therapy compared to individual therapy with Cef alone. To detect the associated molecular pathway, we applied qPCR and found that the combined therapy with ZnONPs and Cef significantly downregulated apoptosis-related genes (*Bax* and caspase 3), inflammation-associated genes (*IL1β* and *TNFα*), and significantly upregulated the anti-apoptotic *Bcl2* gene in MGE cells compared with individual therapy. Finally, oxidative stress was decreased in MGE cells after co-treatment with ZnONPs and Cef, as shown by increased expression of the *NrF2* and *HO-1* genes, decreased levels of MDA, and increased levels of CAT, SOD, and GPx. These findings led us to the conclusion that ZnONP treatment not only reduced the negative effects of Cef on MGE cells, but also boosted the antibiotic's efficacy. Therefore, ZnONPs should be used in conjunction with Cef to treat mastitis especially that caused by *Staphylococcus aureus*.

Keywords: Zinc oxide nanoparticles, cefquinome, buffalo mammary gland epithelial cells, antimicrobial potential.

Introduction

Inflammation of the mammary gland parenchyma, known as mastitis, is a major problem which causes severe losses in dairy production. Mastitis is mainly caused by bacteria in dairy animals and is manifested by a variety of alterations in the milk's chemical, and physical composition. One of the most common bacterial causes of mastitis in dairy animals is *Staphylococcus aureus*, which is non-motile, spores-free, and is resistant to most antibiotics [1]. Extracellular chemicals released by *S. aureus*, such as coagulase, alfa toxin, and enterotoxins, can damage mammary gland epithelial cells. S. aureus is notoriously difficult to eradicate from infected animals [2-8].

Antibiotics are often used for the treatment and prevention of mastitis. Cefquinome (Cef), a fourth-generation cephalosporin, is a common antibiotic used to treat mastitis because of its broad spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria [9]. Cef targets and inactivates penicillin-binding proteins on the bacterial surface [10]. It is highly effective against *S. aureus* [11]. There are, however, drawbacks to

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this treatment approach, such as a lower success rate in curing mastitis, an increase in antibiotic resistance, and the presence of antibiotic residues in milk [12,13]. Some antimicrobials, even at low doses, may prompt *S. aureus* to develop biofilms [14]. Antimicrobial resistance is exacerbated by the presence of biofilm in animals mammary glands [13]. Farm animals treated with antimicrobials may pose a threat to human health. Food-borne pathogens that are resistant to antimicrobials may develop if antimicrobial residues are not removed [10]. Due to its effective pathogenesis, distinctive facultative intracellular parasitism, biofilm development, and developing antimicrobial resistance, *S. aureus*-caused mastitis is very challenging to control with traditional therapy [13].

New approaches are needed for mastitis treatment, such as nanoparticle (NP)-based therapy, since antibiotic treatment is inadequate and causes health concerns in animals and humans. Positive effects have been observed from the use of several NPs in the treatment of mastitis, especially that caused by multi-drug resistant bacteria such as *S. aureus* mastitis [15-17]. Supplementing animals with ZnONPs has been shown to boost growth performance, immunological function, and antioxidant capacity [18]. ZnONPs are able to interact with the surface of bacterial cells, penetrate to their interiors, and display bactericidal processes because of their unique size and shape [19]. Several bacterial cell functions, such as active transport, bacterial metabolism, and enzymes activity, are known to be inhibited by the release of Zn^{2+} from ZnONPs, making this one of the primary assertions in antibacterial processes [20,21]. The production of reactive oxygen species (ROS) is at the root of antibacterial action of ZnONPs, since it is responsible for oxidative stress and the consequent damage or death of bacterium cells [12]. When hydrogen peroxide (H_2O_2) enters a bacterial cell, it damages the cell's lipid, protein, and DNA, ultimately leading to injury and cell death [22]. ZnONPs connect to negatively charged bacterial cells due to their positive zeta potential, allowing them to penetrate [15,16]. This contact may disrupt membrane plasma structure and destroy bacterial cell integrity, leaking intracellular contents and killing the cell [23].

We hypothesized that ZnONPs may cure mastitis and antibiotic-resistant *S. aureus* without harming animal tissues. Therefore, this study aimed to investigate whether ZnONPs would boost Cef antibacterial potential and reduce its side effects on buffalo mammary gland epithelial cells.

Materials and Methods

Materials

Zinc oxide nanoparticles (ZnONPs) (Purity 98.8%, Size 30 nm) was purchased from AEM®, Changsha, Hunan, China), Mueller-Hinton agar (MH) culture medium from Oxoid, Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) (Cat. no.10099133) from GIBCO, MTT from Molecular Probes, dimethyl sulfoxide (DMSO) from Sigma, Trizol from Invitrogen, RevertAid H Minus Reverse kit and Syber Green from Thermo Scientific.

Milk Samples

In this investigation, we utilized a total of 12 milk samples from 12 lactating buffaloes with clinical mastitis. Before milk samples were taken, the udder teats were sanitized with ethyl alcohol at 70%. Milk samples were collected in sterile tubes and transported on ice to the lab. Each tube contained 10 ml of milk. Within 2 hours of sample collection, a bacteriological analysis was conducted.

Bacteria Isolation and Characterization

The milk samples were cultured on mannitol salt agar for *S. aureus* isolation. After 24 hours of incubation at 37°C, Gram staining and biochemical assays (catalase, DNase, and coagulase tests) were performed on the cultures.

Agar Well Diffusion Assay for Determining ZnONPs and Cef's Antibacterial Activity

Using MH culture media (agar well diffusion technique), we measured the ZnO-NPs' and Cef's antibacterial efficacy. Bacteria were freshly cultured, then transferred into nutrient broth with a turbidity of 0.5 to meet the McFarland standard. Bacterial suspensions were plated out on MH agar medium in Petri dishes, and then 5 mm diameter wells were put on top of the test organisms and infused with varying doses of ZnONPs or Cef (1, 3, 5, 10, 20, 30, 40, and 50 mg/ml). After incubating the Petri dishes at 37°C for 24 hours, the antibacterial activity was determined by measuring the size of the inhibition zones surrounding the wells.

Calculation of ZnONPs and Cef's MIC and MBC

The minimum inhibition concentration (MIC) was calculated using the microdilution technique in 96-well microplates. Briefly, a total of 95 µl MH broth medium and 5 µl of bacterial inoculum were evenly distributed throughout the 96 wells of a 96-well plate. The wells were then filled with 100 µl ZnONPs or Cef solution (1-50 mg/ml). After that, the microplates were kept in a 37°C incubator for 24 hours. Microplate reader absorbance readings at 600 nm were used to calculate growth rates. Each well's contents (200 ml) was transferred to an Eppendorf tube containing 2 ml of BHI medium, and the tube was incubated at 37°C for 12 hours to see whether the bacteria were killed. The cultures without turbidity were then sampled, and 20 ml

were inoculated onto BHI agar and incubated at 37°C for 24 hours. The lack of bacterial colonies on an agar plate was used to determine the amounts of ZnONPs or Cef with bactericidal properties. The minimal inhibitory concentration (MBC) was determined by comparing the amount of ZnONPs or Cef required to completely halt bacterial growth.

Isolation of Mammary Gland Epithelial Cells

Isolation of buffalo mammary gland epithelial cells (MGE) was performed as previously detailed [24]. To separate the fat and cell fractions from buffalo milk sample (350 ml), centrifugation at 1500 rpm for 1 h was applied. After a second centrifugation at 1250 rpm for 20 minutes, the obtained cell pellet was rinsed twice with phosphate buffer saline (PBS). A suspension of the filtered pellet containing MGE cells was cultured in Dulbecco's Modified Eagle's Medium (DMEM, GIBCO) at 37°C in a CO2 incubator for 24 h until reaching a confluence of 70-80%.

The MTT Assay

MGE cells were subjected to the MTT assay to determine the impact of ZnONPs and/or cefquinome on the cell viability using a previously published procedure [25]. In brief, in DMEM with 10% heat-inactivated FBS, 10000 cells were seeded into each well. After incubating the cells for 24 hours at 37°C, MGE cells were treated with Cef at doses of 0, 12.5, 25, 50, 100, 200, and 400 µg/ml or with ZnONPs at concentrations of 0, 2.5, 5, 10, 15, 20, and 25 µg/ml. Following a 4-hour re-incubation at 37°C, cells were treated with 12 mM (10 µl per well) MTT. At last, after 30 min, we discarded the MTT solution and replaced it with 100 µl of DMSO. The IC_{50} was determined by measuring the absorbance at 570 nm and then plotting a sigmoidal curve in GraphPad Prism 8.

Experimental groups

Four groups of MGE cells were randomly created. Cells treated with a placebo (DMSO) were used as a control group (Cnt group), while other cells were treated with 25 µg/ml Cef (Cef group) and 25 µg/ml ZnONPs (ZnONPs group) each alone or in combination (Comb group). These doses were used based on the IC_{50} value of Cef. Once the MGE cells had attained confluence after 24 h incubation, we used them for RNA isolation and biochemical assays.

Real time PCR (qPCR)

The qPCR was used to detect the altered expression of apoptosis-related genes (*Bax*, caspase3, and *Bcl2*), inflammation-associated genes (*IL1β* and *TNFα*), and antioxidant-related genes (*NrF2*, and *HO-1*) genes in MGE cells. We began by using Trizol to extract total RNA from MGE cells. and detected RNA concentration and purity using a Nanodrop [26,27]. Next, we got cDNA after converting the RNA using reverse transcriptase [28,29]. Piko qPCR thermal cycler (Thermo Scientific) and its integrated software were used for the qPCR test and data processing, respectively. Syber Green was added in the PCR mixture with cDNA and primers. The primers were designed based on the previously published articles [30,31]. We kept the temperature within the range recommended by the manufacturer as previously described [32]. The $2^{-\Delta\Delta Ct}$ formula was used to quantify the fold change in gene expression relative to the housekeeping gene *B-actin*.

Oxidant and antioxidant parameters

We used commercially available kits (Biodiagnostic, Cairo, Egypt) to measure lipid peroxide malondialdehyde (MDA) levels and the activities of the antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in the MGE cells following all treatments in accordance with our previous description [33,34]. To calculate MDA, we first determined the concentration of thiobarbituric acid reactive compounds (TBARS). In an acidic environment, TBA reacted with MDA to generate TBARS. At a pH of 10.2, SOD prevents epinephrine from being converted to adrenochrome. The rate of H_2O_2 dissociation at 240 nm was used to compute CAT activity.

Statistical analysis

GraphPad Prism 8.0 was used for statistical analysis (San Diego, CA). Significant differences between groups were determined using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Statistical significance was determined using a value of $P < 0.05$, and the data was shown as a mean±SEM.

Results

ZnONPs Boosted Antimicrobial Potential of Cef Against

S. aureus

Gram staining, biochemical tests, catalase, DNase, and coagulase activity assays, and observation of yellow colonies on mannitol salt agar confirmed the isolation of *S. aureus* in 6 of 12 samples. Antibacterial activity against *S. aureus* was enhanced when ZnONPs and/or Cef were used together, with a maximal zone of inhibition at a dose of 50 mg/ml reaching 3.7 mm. However, individual treatment with ZnONPs or Cef resulted in zone of inhibition reaching 1.2 or 3.0 mm, respectively. Again, the combined treatment showed higher antibacterial activities as revealed by MIC and MBC values for *S. aureus* which were 0.15 µg/ml and 0.30 µg/ml, respectively. In contrast, individual treatment showed lower

antibacterial activities. MIC and MBC values for *S. aureus* were 6.25 µg/ml and 8.50 µg/ml, respectively, with ZnO-NPs and were 0.25 µg/ml and 0.5 µg/ml, respectively, with Cef.

ZnONPs Reduced Cytotoxic Potential of Cef on MGE Cells

The MTT assay was applied to assess the cytotoxic capability of ZnONPs and/or Cef on buffalo MGE cells (Fig. 1). The IC_{50} value for Cef's cytotoxic effects on MGE was 53.25±3.40 µg/ml. However, at concentrations greater than 5 µg/ml (10 - 20 µg/ml), ZnONPs exhibited no harmful effects. Interestingly, cell viability was significantly ($P \leq 0.05$) increased by 25 µg/ml ZnONPs compared to control (untreated) cells. Cells co-treated with ZnONPs and Cef had significantly ($P \leq 0.05$) higher cell viability than cells treated with Cef alone as revealed by higher IC_{50} value of 521.38±25.66 µg/ml.

ZnONPs Inhibited Apoptosis Induced by Cef on MGE Cells

As shown in Figure 2, qPCR data revealed a significant ($P \leq 0.05$) upregulated expression of the apoptotic genes *Bax* and caspases and a significant ($P \leq 0.05$) downregulated expression of the anti-apoptotic *Bcl2* gene in MGE cells treated with Cef alone compared to the control (untreated) cells. In contrast, cells treated with ZnONPs exhibited significantly ($P \leq 0.05$) reduced expression of *Bax* and caspase 3 expression and significantly ($P \leq 0.05$) increased expression of *Bcl2* relative to all other groups. Moreover, cells cotreated with ZnONPs and Cef denoted significantly ($P \leq 0.05$) lower *Bax* and caspase3 expression than cells treated with Cef alone. On the other hand, no significant results were obtained in cells co-treated with ZnONPs+Cef and control cells.

ZnONPs Decreased Inflammation Induced by Cef on MGE Cells

MGE cells treated with Cef alone denoted significantly ($P \leq 0.05$) elevated mRNA levels of the two inflammatory genes (*IL1β* and *TNFα*) relative to the control (untreated) cells (Fig. 3). However, MGE cells treated with ZnONPs alone or in combination with Cef showed significantly ($P \leq 0.05$) declined *IL1β* and *TNFα* expression, with lowest expression in ZnONPs-treated cells, compared to cells treated with Cef alone. However, ZnONPs alone or in combination with Cef did not restore expression levels to those seen in the control group (Fig. 3).

ZnONPs Suppressed Oxidative Stress Induced by Cef on MGE Cells

Figures 3 and 4 show the effect of treatment with ZnONPs and/or Cef on oxidative and antioxidant status in MGE cells. Treatment of MGE cells with Cef alone resulted in a significant ($P<0.05$) increase in MDA levels and a significant ($P<0.05$) decrease in activity of the antioxidant enzymes (SOD, CAT, and GPx) and the expression of antioxidant-related genes (*NrF2* and *HO-1*) as compared to all other cells. In contrast, cells treated with ZnONPs alone or in combination with Cef exhibited significantly ($P \leq 0.05$) decreased MDA levels and significantly ($P \leq 0.05$) increased SOD, CAT, and GPx levels and upregulated expression of *NrF2* and *HO-1*, with best effects in ZnONPs-treated cells, compared to cells treated with Cef alone.

Discussion

Treating mastitis caused by *S. aureus* is notoriously difficult because of the bacteria's propensity to rapidly build biofilms and resistance to most antibiotics, including Cef [1-8,12,13]. Several NPs have been shown to be effective in the treatment of mastitis, particularly mastitis caused by multi-drug-resistant bacteria such as S. *aureus* [15-17]. ZnONPs showed potent antibacterial potential against S. *aureus* isolated from cow milk [35]. However, little is known whether ZnONPs would boost the anti S. *aureus* effects of Cef and ameliorate its side effects on buffalo MGE cells. Therefore, we conducted this study to check this effect. To the best of our knowledge, this is the first study to report that ZnONPs treatment not only decreased the side effects of Cef on MGE cells, but also increased its antibiotic's potential against *S. aureus* isolated from milk of mastitic buffaloes.

Our results confirmed by those obtained by Emami-Karvani and Chehrazi [36] and Hozyen, et al. [35] showed that ZnONPs demonstrated strong antibacterial activity at doses of 1-50 mg/ml against *S. aureus* isolated from buffaloes and cows with clinical mastitis. The effectiveness of NPs as an antibacterial agent is significantly influenced by their concentration [37] since they release so many ions when their numbers are high [38]. Furthermore, we and Wahab et al. [39] found that higher concentrations of ZnONPs in discs led to a greater inhibition zones of bacterial growth. We also found that ZnONPs were effective against *S. aureus*, as shown by the MBC and MIC values. The findings of Paredes et al. [40] on the sensitivity of *S. aureus* to silver nanoparticles are consistent with our results. In contrast, Gram-positive bacteria have been shown to be more resistant to the modes of action of ZnONPs due to their thick peptidoglycan coating, which is composed of teichoic and teichuronic acids that are covalently bonded to the peptidoglycan [35,36]. This conflict in results could be attributed to the size of ZnONPs used in the *in vitro* study. We used a very small size which is 30 nm while other studies used a very larger size above 100 nm.

Cef exerted higher anti *S. aureus* effects than ZnONPs. However, co-treatment with ZnONPs and Cef induced higher anti *S. aureus* effects. Cef targets and inactivates penicillin-binding proteins on the bacterial wall [10] and is highly effective against *S. aureus* [11]. However, the processes by which ZnONPs inhibit bacterial growth are a matter of some contention. ZnONPs are able to interact with the surface of bacterial cells, penetrate to their interiors, and display bactericidal processes because of their unique size and shape [19]. ZnONPs are 250 times smaller than a bacterium, and can connect with negatively charged bacterial cell wall, allowing them to either destroy the cell wall or penetrate within the cells and release Zn^{2+} that inhibits many bacterial cell activities, including active transport, bacterial metabolism, and enzymes, thereby leading to death of bacteria [15,16,20,21,23,41]. Another possible mode of antibacterial potential of ZnONPs is the induction of ROS that is responsible for oxidative stress and the consequent damage or death of bacteria [12]. ZnONPs induce free radicals release such as H_2O_2 that damages the cell's lipid, protein, and DNA, ultimately leading to cell death [22]. The great effectiveness of the combined treatment with ZnONPs and Cef against *S. aureus* could be due to the activation of numerous antibacterial pathways at once. ZnONPs and Cef, when taken together, have the potential to block many targets in *S. aureus* at once, making them effective antimicrobial agents against mastitis caused by *S. aureus*.

To the best of our knowledge, this is the first study to report significantly lowered cytotoxic effects for the combined therapy with ZnONPs and Cef on buffalo MGE cells compared to individual therapy with Cef alone as revealed by the MTT assay. To detect the associated molecular pathway, we applied qPCR on mRNA isolated from MGE cells and found that the combined therapy with ZnONPs and Cef significantly downregulated apoptosis-related genes (*Bax* and caspase 3), and significantly upregulated the anti-apoptotic *Bcl2* gene compared with individual therapy. Finally, oxidative stress was decreased in MGE cells after co-treatment with ZnONPs and Cef, as shown by increased expression of the *NrF2* and *HO-1* genes, decreased levels of MDA, and increased levels of CAT, SOD, and GPx. These benificial effect could be attributed to the anti-apoptotic, and anti-oxidant of the Zn. In support, supplementing animals with ZnONPs has been shown to boost growth performance, immunological function, and antioxidant capacity [18]. Similarly, ZnONPs also dose-dependently decreased the lipid peoxide marker MDA [42]. Zinc is essential for the body's metabolism and is involved in processes including hematopoiesis, enzyme control, cellular redox balance, and the regulation of DNA and protein production machinery [43]. The Food and Drug Administration (FDA) has determined that ZnONPs are safe GRAS materials [18]. When compared to other metal nanoparticles, ZnONPs are the most promising because of their exceptional anti-apoptotic, anti-oxidant and anti-inflammatory characteristics [44]. ZnONPs is a promising drug delivery medium because of the additive benefits it has when used with conventional medications [45].

In the present study, we found that MGE cells treated with ZnONPs had significantly lower expression of inflammation-associated genes (*IL1β* and *TNFα*). Consistence with our results, similar anti-inflammatory potential was recorded by other studies [20,46,47]. ZnONPs block NF-κB pathways through inhibition of IL1β, TNFα, and COX2 cytokines [17,47]. There was also a dose-dependent decrease in NO generation by activated macrophages when ZnONPs was added [48]. Thus, inhibition of the release of pro-inflammatory cytokines could be a common anti-inflammatory strategy used by ZnONPs. Proteins such as IgG, IgM, and fibrinogen are often seen following NP exposure [47] and could participated in the anti-inflammatory process [49] through the activation of the anti-inflammatory M2 macrophages [22]. Inflamed mucosal tissue was the target of NP deposition and adsorption [50].

Conclusions

This is the first work to our knowledge to show that treatment with ZnONPs not only mitigated Cef's toxic effects on MGE cells but also boosted the antibiotic's efficacy against *S. aureus* isolated from the milk of mastitic buffaloes.

Conflicts of interest:

"There are no conflicts to declare".

Funding statement:

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Author`s contributions:

"Authors contribute equally in this work"

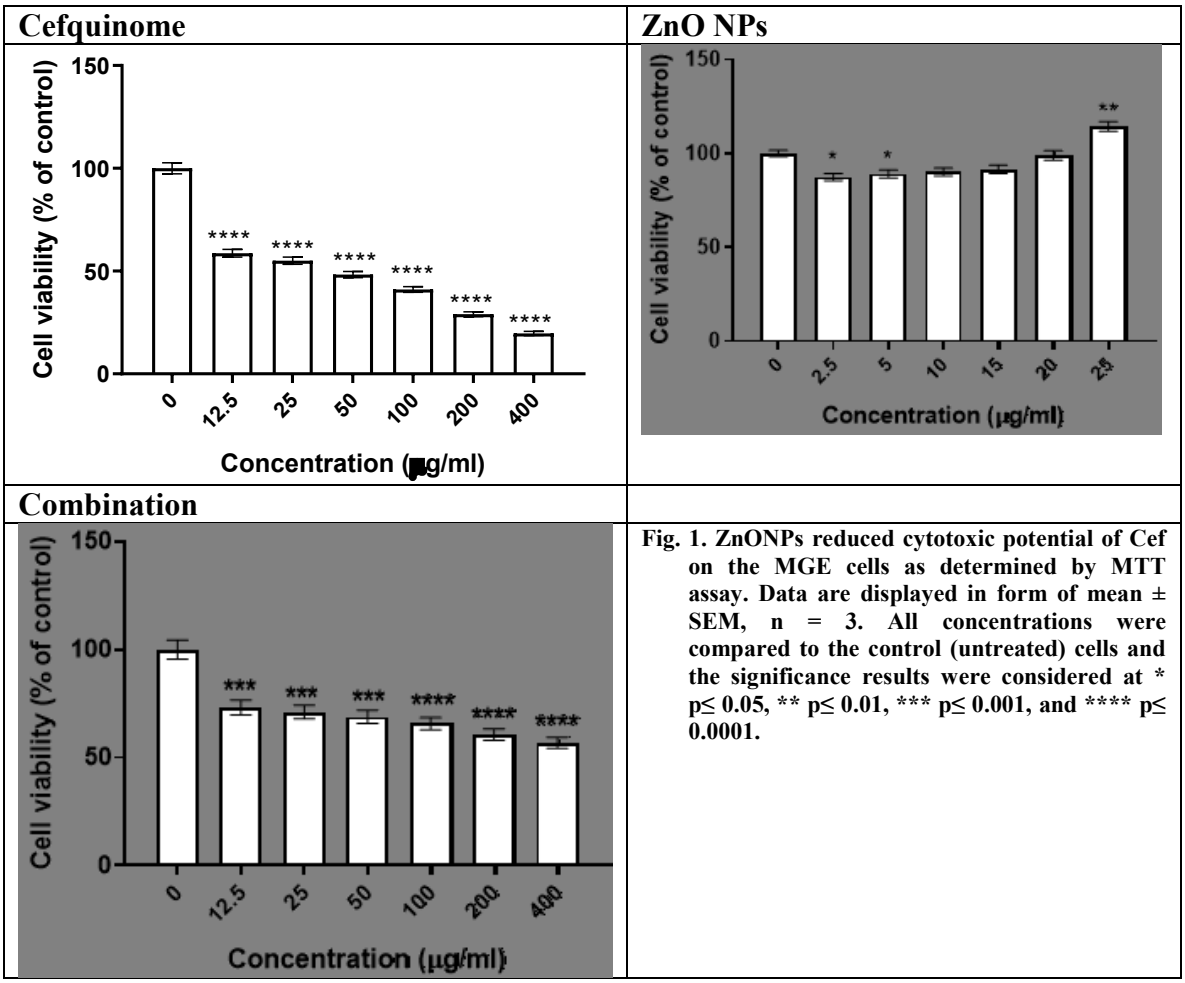

Fig. 1. ZnONPs reduced cytotoxic potential of Cef on the MGE cells as determined by MTT assay. Data are displayed in form of mean ± SEM, n = 3. All concentrations were compared to the control (untreated) cells and the significance results were considered at * p≤ 0.05, ** p≤ 0.01, *** p≤ 0.001, and **** p≤ 0.0001.

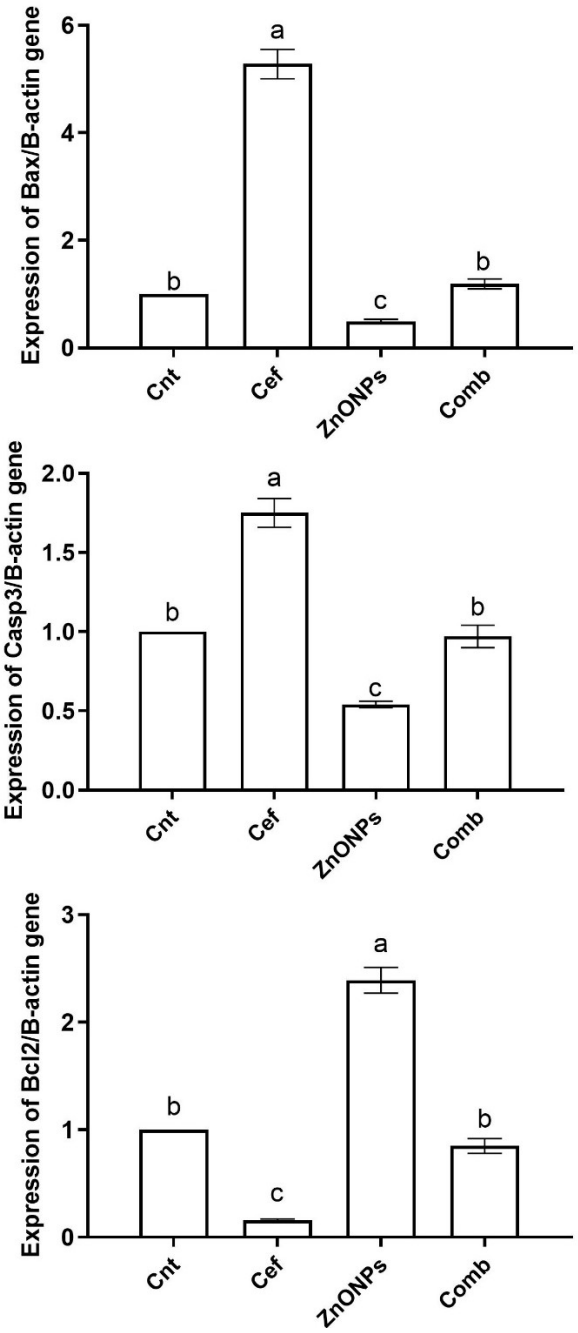

Fig. 2. ZnONPs inhibited apoptosis induced by Cef on the MGE cells as determined by altered expression of *Bax*, caspase 3, and *Bcl2* genes detected by qPCR. The relative gene expression was presented as mean ± SEM (5 replicates per group). Columns (means) and error bars (SEM) had different letters (a – c) indicate statistical differences at $p < 0.05$. All groups were compared to each other. Cnt, untreated control cells, Cef, cefquinome-treated cells, ZnONPs, zinc oxide nanoparticles-treated cells, Comb, cefquinome + zinc oxide nanoparticles-treated cells.

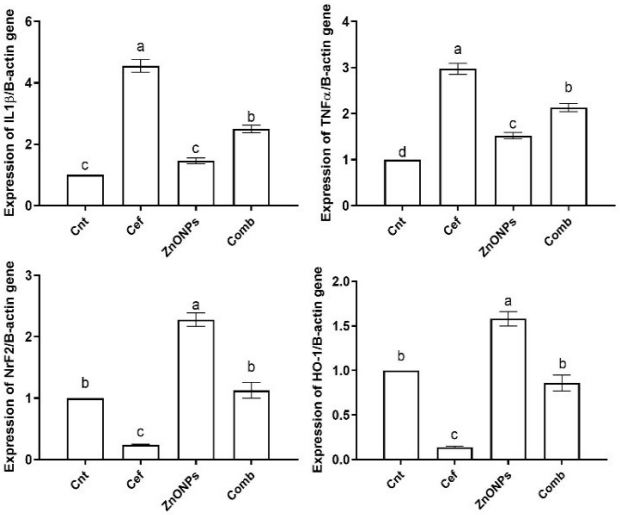

Fig. 3. ZnONPs decreased inflammation and oxidative stress induced by Cef on the MGE cells as determined by altered expression of *IL1β*, *TNFα*, *NrF2*, and *HO-1* genes detected by qPCR. The relative gene expression was presented as mean ± SEM (5 replicates per group). Columns (means) and error bars (SEM) had different letters (a – c) indicate statistical differences at $p < 0.05$. All groups were compared to each other. Cnt, untreated control cells, Cef, cefquinome-treated cells, ZnONPs, zinc oxide nanoparticles-treated cells, Comb, cefquinome + zinc oxide nanoparticles-treated cells.

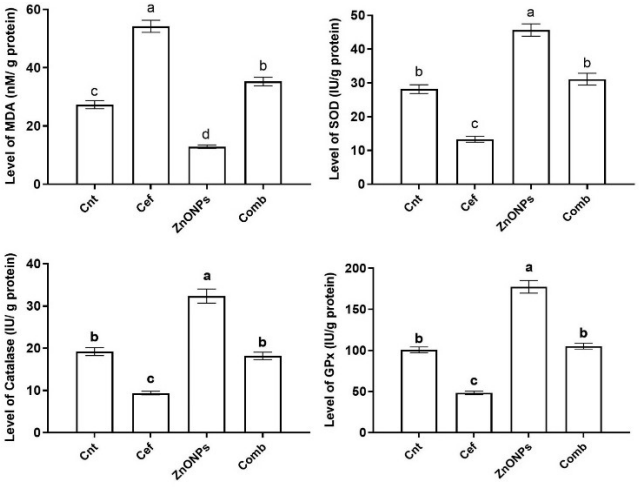

Fig. 4. ZnONPs reduced oxidative stress induced by Cef on the MGE cells. Data was presented as mean ± SEM (5 replicates per group). Columns (means) and error bars (SEM) had different letters (a – d) indicate statistical differences at $p < 0.05$. All groups were compared to each other. Cnt, untreated control cells, Cef, cefquinome-treated cells, ZnONPs, zinc oxide nanoparticles-treated cells, Comb, cefquinome + zinc oxide nanoparticles-treated cells.

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عززت جسيمات أكسيد الزنك النانوية فعالية مضادات الميكروبات في السيفكوينوم وخففت من آثارها الجانبية على خلايا الغدة الثديية فى الجاموس

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هدفت هذه الدراسة إلى التحقق مما إذا كانت جسيمات أكسيد الزنك النانوية (ZnONPs) ستخفف من الآثار الجانبية للسيفكوينوم (Cef) على خلايا الغدة الثديية فى الجاموس (MGE) وتزيد من إمكاناتها المضادة للبكتيريا. قمنا أولا بتقييم التأثير المضاد للبكتيريا ل ZnONPs و / أو Cef ووجدنا إمكانات مضادة لجراثيم المكورات العنقودية الذهبية المعزولة من حليب الضرع أعلى بكثير للعلاج الثنائى .بعد ذلك ، استخدمنا اختبار MTT لتقييم التأثيرات السامة ل ZnONPs و / أو Cef على خلايا MGE ووجدنا تأثيرات سامة للخلايا منخفضة بشكل كبير للعلاج المشترك مقارنة بالعلاج الفردي باستخدام Cef وحده. للكشف عن المسار الجزيئي المرتبط ، قمنا بتطبيق qPCR ووجدنا أن العلاج المشترك مع ZnONPs و Cef قلل بشكل كبير من الجينات المرتبطة بموت الخلايا المبرمج (Bax و caspase 3) ، والجينات المرتبطة بالالتهاب (IL1β و TNFα) ، و قام بزيادة التعبير الجينى ل Bcl2 المضاد لموت الخلايا المبرمج بشكل كبير مقارنة بالعلاج الفردي. أخيرا ، انخفض الإجهاد التأكسدي في خلايا MGE بعد المعالجة المشتركة ب ZnONPs و Cef ، كما يتضح من زيادة التعبير عن جينات NrF2 و HO-1 ، وانخفاض مستويات عامل التاكسد الدهنى MDA ، وزيادة مستويات الانزيمات المضادة للاكسدة CAT و SOD و GPx. قادتنا هذه النتائج إلى استنتاج مفاده أن علاج ZnONP لم يقلل فقط من الآثار السلبية ل Cef على خلايا MGE ، ولكنه عزز أيضا فعالية المضادات الحيوية. لذلك ، ي,وصى باستخدام ZnONPs جنبا إلى جنب مع Cef لعلاج التهاب الضرع وخاصة الناتج عن الاصابة ب جراثيم المكورات العنقودية الذهبية.

الكلمات المفتاحية: جسيمات أكسيد الزنك النانوية ، السيفكوينوم ، الخلايا الظهارية للغدة الثديية الجاموسية ، إمكانات مضادات الميكروبات