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In-Vivo Assessment of Ciprofloxacin and Meropenem-Loaded Solid Lipid Nanoparticles as a Promising Antimicrobial Nano-drug Delivery System against *Pseudomonas aeruginosa* in Broiler Chicks

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Abstract

This study evaluated the in-vivo efficacy of orally administered solid lipid nanoparticles (SLNPs), either alone or loaded with ciprofloxacin or meropenem, as novel nano-drug delivery systems in broiler chicks experimentally infected with *Pseudomonas aeruginosa*. The therapeutic effects of these formulations on growth performance, immunological biomarkers, and the expression of immune-modulatory and antioxidant-related genes were assessed and compared to conventional antibiotic therapy. The results demonstrated that both nano-drug formulations exerted superior therapeutic efficacy, with recovery rates of 79% and 75.8% for SLNPs-CIP and SLNPs-MEM, respectively. Treatment with SLNPs-CIP significantly reduced inflammatory responses, as evidenced by a 52.3% and 52.2% decrease in hepatic and ileal TNF-α concentration, respectively, and markedly enhanced antioxidant defence, with SOD activity increasing by 178.4% in the liver and 189.8% in the ileum. At the molecular level, SLNPs-CIP down-regulated TNF-α gene expression by 75.5% (liver) and 84.1% (ileum), and up-regulated SOD gene expression by 188% and 481.3%, respectively. These molecular and biochemical modulations were accompanied by notable histopathological improvements in liver and intestinal tissues. Overall, the findings highlight the superior performance of SLNP-based drug delivery, particularly SLNPs-CIP, as a promising nanotherapeutic approach for controlling *P. aeruginosa* infections in poultry by improving drug bioavailability, prolonging antibacterial effects, and mitigating tissue damage.

Keywords: Ciprofloxacin, Drug delivery, Meropenem, *Pseudomonas aeruginosa*, Solid lipid nanoparticles.

Introduction

The treatment of intracellular bacterial infections, such as those caused by *Pseudomonas aeruginosa*, poses significant challenges to poultry health and productivity due to the increasing prevalence of antibiotic resistance. The effectiveness of conventional antibiotics is often compromised by enzymatic degradation within lysosomes and limited cellular penetration, which reduces their therapeutic efficacy. As a result, there is a growing need to develop alternative drug delivery strategies capable of enhancing the targeting and bioavailability of antimicrobial agents [1].

Nanotechnology offers innovative solutions in this regard. Nanoparticles have emerged as promising drug delivery vehicles capable of transporting both low-molecular-weight compounds and macromolecules including proteins, peptides, and genes to specific cells and tissues, while protecting them from enzymatic degradation. Nano-drug delivery systems enhance therapeutic efficacy by improving drug solubility, cellular uptake, and controlled release profiles, thereby minimizing systemic side effects [2].

Among various nano-particulate systems, solid lipid nanoparticles (SLNPs) stand out due to their unique physicochemical and biological properties.

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These include biocompatibility, long-term stability, low toxicity, ability to encapsulate both hydrophilic and lipophilic drugs, and suitability for oral administration. SLNPs offer protection against gastrointestinal degradation and facilitate targeted, sustained drug release at the site of infection [1, 3]. Their non-immunogenic and biodegradable nature further enhances their potential as drug carriers in veterinary and human medicine [4, 5].

Previous studies have demonstrated the effectiveness of SLNPs loaded with various antibiotics in treating chronic bacterial infections. These drug-loaded nanoparticles can enter infected cells *via* phagocytosis, enabling the accumulation of therapeutic agents within macrophages, which is critical for eliminating intracellular pathogens. Their slow drug release profile further ensures prolonged antibacterial action, reducing the likelihood of disease progression in immunocompromised hosts [1, 6].

Pseudomonas aeruginosa is a highly opportunistic Gram-negative bacterium known to cause severe infections in both animals and humans. It is commonly found in water, soil, and moist environments [7, 8]. In poultry, *P. aeruginosa* infections can result in high morbidity and mortality, especially in young birds, due to conditions such as septicemia, respiratory infections, sinusitis, keratoconjunctivitis, and embryonic death in hatcheries. In humans, the pathogen is a public health concern, often transmitted through occupational exposure to contaminated poultry products [9-11].

Ciprofloxacin (CIP) is a broad-spectrum fluoroquinolone antibiotic effective against a wide range of Gram-positive and Gram-negative bacteria, including *P. aeruginosa* [12]. Meropenem (MEM), a β-lactam antibiotic belonging to the carbapenem class, is also widely used due to its broad antimicrobial spectrum and low toxicity, making it an essential option for treating *P. aeruginosa* infections resistant to conventional therapies [13].

We hypothesize that SLNPs loaded with ciprofloxacin and meropenem will improve drug bioavailability, reduce inflammation and oxidative stress, and promote tissue recovery better than free antibiotics, providing a safer and more effective therapeutic approach. So, the present study aimed to evaluate the efficacy of ciprofloxacin- and meropenem-loaded SLNPs as nanodrug delivery systems for the treatment of *P. aeruginosa* infections in broiler chicks. The study focused on assessing clinical, biochemical, genetic, and histopathological outcomes to determine the therapeutic potential of these nanoparticle-based formulations.

Material and Methods

Materials

Ciprofloxacin and meropenem were used as model antibiotics in this study. Solid lipid nanoparticles (SLNPs) were prepared and utilized either alone (5%) or as carriers for the antibiotics. A virulent *Pseudomonas aeruginosa* strain, previously isolated and characterized at the Faculty of Veterinary Medicine, Mansoura University, was used for infection. A total of seventy Pseudomonas-free one-day-old Hubbard broiler chicks were sourced from a commercial hatchery. Phosphate-buffered saline (PBS, 0.1 M, pH 7.6) was used for tissue homogenization. For gene expression analysis, Trizol reagent, Direct-zol™ RNA MiniPrep kits, SensiFast™ cDNA synthesis and SYBR Green PCR kits were employed. TNF-α and SOD levels were quantified using commercial enzymatic colorimetric kits. Histological examinations were performed using standard hematoxylin and eosin (H&E) staining procedures. All the bio-chemicals and drugs were purchased from Sigma Aldrich, Germany. All the inorganic chemicals (analytical grade) were obtained from various local producers and suppliers.

Experimental Design

A field experiment was conducted at a private poultry farm located in Meet-Khamis village, Dakahlia Governorate, Mansoura, Egypt, from early November to late December 2023. Seventy one-day-old *Pseudomonas*-free Hubbard broiler chicks were obtained from a commercial hatchery and randomly assigned to seven experimental groups (n = 10 per group):

Group 1: Healthy control (non-infected, untreated)
Group 2: Infected control (challenged with *P. aeruginosa*, untreated)
Group 3: Infected + treated with meropenem (160 µg/mL)
Group 4: Infected + treated with ciprofloxacin (0.65 µg/mL)
Group 5: Infected + treated with SLNPs alone (5%)
Group 6: Infected + treated with SLNPs loaded with meropenem (5% SLNPs + 160 µg/mL MEM)
Group 7: Infected + treated with SLNPs loaded with ciprofloxacin (5% SLNPs + 0.65 µg/mL CIP)

All chicks were housed in disinfected pens, monitored over a 6-week (experimental period), and provided a non-medicated broiler diet and had free access to water and feed. Diets were formulated in accordance with NRC [14].recommendations (Table 1).

Infection and Treatment Protocol

At 14 days of age, chicks in all infected groups received a 1 cm^3 oral inoculation of *Pseudomonas aeruginosa* suspension (1×10^9 CFU/mL) *via* syringe with polyethylene tubing [15]. Healthy controls were kept in separate enclosures to avoid cross-contamination. At 25 days of age, post-clinical manifestation, treatments were administered orally. Room temperature was maintained at 32 °C initially and gradually reduced to 25 °C by the end of the experiment. The *P. aeruginosa* strain used was previously isolated and characterized in the

Microbiology Laboratory, Faculty of Veterinary Medicine, Mansoura University [16]. All experimental protocols were approved by the Mansoura University Animal Care and Use Committee (MU-ACUC).

Monitoring of External Clinical Signs and Mortality

Chicks were observed twice daily for clinical signs and mortality throughout the experimental period.

Growth Performance

Body weights were recorded at days 7, 14, 24, and 35 to assess the impact of treatment on growth performance [17].

Biochemical Analysis

Liver and intestinal samples (~0.5 g) were homogenized in 5 cm^3 phosphate-buffered saline (0.1 M, pH 7.6), centrifuged at 500 ×g for 10 minutes, and the supernatant was analyzed for TNF-α and SOD using enzymatic colorimetric kits [18, 19].

Gene Expression Analysis (RT-qPCR)

At the end of the experimental period, three birds from each group were euthanized then liver and intestinal tissues were collected, washed in phosphate buffer solution (PBS), snap-frozen in liquid nitrogen, and stored at –80 °C. Total RNA was extracted using Trizol reagent (Direct-zol™ RNA MiniPrep, R2050), and RNA quality was assessed using a Nanodrop spectrophotometer and gel electrophoresis.
First-strand cDNA was synthesized using the SensiFast™ cDNA synthesis kit (Bio-65053). Quantitative real-time PCR was performed using SensiFast™ SYBR Green PCR Master Mix (Bio-98002). Reactions were run in 20 µL volumes with the following conditions: initial denaturation at 95 °C for 2 min, followed by 40 cycles of 94 °C for 15 s, gene-specific annealing (Table 2) for 30 s, and 72 °C for 20 s. Melt curve analysis was used to verify amplification specificity. Gene expression levels were calculated using the 2^−ΔΔCt method [20] with β-actin as the housekeeping gene.

Histopathological Evaluation

Liver and intestinal samples were fixed in 10% neutral-buffered formalin for 24 hours, embedded in paraffin, sectioned at 5 µm using a rotary microtome, and stained with hematoxylin and eosin (H&E). Tissue morphology was examined under a light microscope (Olympus CX31) as described by Bancroft and Gamble [21].

Statistical Analysis

All experiments were performed in triplicate. Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test using SPSS software version 17.0 (SPSS Inc., Chicago, IL). Results were considered statistically significant at $p < 0.05$.

Results

External Clinical signs and mortality rate

External clinical signs became apparent 7 days post-infection. In this respect, the infected chicks were suffered from weakness, diarrhoea, localized infections in different organs, loss of appetite, depression, closed eye, respiratory manifestations and keratoconjunctivitis during the period from 14 to 24 d-old as recorded in table 3. The using of both CIP and MEM either singly or loaded on SLNPs as nano-drug delivery systems reduced the severity of the appeared clinical signs when compared with infected group. No mortalities were recorded in groups throughout the experimental period.

Growth Performance

Perusal of data in table 4 revealed that oral administration of ciprofloxacin, meropenem, and SLNPs either singly or in combination significantly enhanced the growth and increased final body weight of the experimental infected chicks treated with these strategies as compared to the infected group. The most prominent increase in the body weight was observed in SLNPs-CIP treated group by 79%. These improvements reflect better nutrient utilization and reduced systemic burden. The following sequence of treatment: SLNPs-CIP> SLNPs-MEM > SLNPs > CIP > MEM was display with respect to control (infected or healthy) chicks.

Changes in biochemical indices

Data pertaining to the antioxidant and immune indicators in the chicken liver and intestine are presented in table 5. Nano-drug delivery systems especially SLNPs-CIP resulted in a marked reduction in TNF-α levels, with approximately 52% decrease in both hepatic and intestinal tissues compared to the infected group. In contrast, the activity of superoxide dismutase (SOD), a key antioxidant enzyme, exhibited a significant increase in the treated groups, indicating an enhanced anti-oxidative response. It is of interest to mention that the percentage recovery of both TNF-α concentration and SOD activity of the variously treated chickens either healthy or recovered from illness, showed variable decreases above the control infected level as shown in table 5 and 6. Additionally, the radical scavenging effect of the used antibiotics was enhanced with nanosoild lipid systems, as evinced by reducing the ROS production during spontaneous oxidative stress indicating effective modulation of inflammation and oxidative stress.

Changes in gene expression

The results pertaining to the gene expression profile of activity of selected antioxidant enzymes (SOD) and immune modulator (TNF-α) markers in

liver and intestine of experimentally chicks is shown in table 7 and 8. Notably, treatment with ciprofloxacin (CIP), meropenem (MEM), and solid lipid nanoparticles (SLNPs), whether administered individually or in combination, resulted in a significant down-regulation of TNF-α expression in both liver and intestinal tissues compared to the infected untreated group. The most pronounced reduction was observed in the SLNPs-CIP treatment group, with TNF-α expression levels reduced by 72.8% in the liver and 28.4% in the intestine relative to the infected control group. Conversely, the expression pattern of SOD demonstrated a marked upregulation in treated groups, with SLNPs-CIP-treated chicks exhibiting the highest levels of hepatic and intestinal SOD expression. This indicates an enhanced antioxidative response facilitated by the nanodrug delivery system.

The calculated percent recovery and percent change in gene expression levels of TNF-α and SOD in both hepatic and intestinal tissues following treatment with SLNPs either unloaded or loaded with ciprofloxacin or meropenem are presented in Tables 7 and 8. These results demonstrate that SLNPs-CIP was particularly effective in restoring normal gene expression levels, suggesting its superior therapeutic efficacy compared to other treatment modalities.

Histopathological evaluation of Liver and Intestine

Representative photomicrographs of hepatic sections from the various treatment groups are shown in Figure 1. The control group exhibited normal hepatic architecture, with hepatocytes arranged radially around a central vein (Fig. 1A). In contrast, the infected group showed extensive pathological alterations characterized by granulomatous hepatitis with massive infiltration of macrophages, lymphocytes, and occasional neutrophils, resulting in near-complete replacement of hepatic parenchyma (Fig. 1B). Additional histological features included diffuse parenchymal replacement by macrophages and lymphocytes (thin arrow), necrotic hepatocytes, and sinusoidal congestion (arrowhead) (Fig. 1C), as well as ballooning degeneration (thick arrow) and microvesicular steatosis (thin arrow) (Fig. 1D).

Liver sections from MEM-treated chicks demonstrated mild sinusoidal congestion and focal necrosis surrounded by sparse macrophage infiltration (Fig. 1E). The CIP-treated group exhibited focal hepatic necrosis (thick arrows) surrounded by lymphocytes, macrophages, neutrophils, and eosinophils (thin arrow) (Fig. 1F). Treatment with SLNPs alone resulted in focal, coalescing periportal aggregates of macrophages and lymphocytes (thin arrow), with occasional hepatocyte necrosis in the surrounding tissue (thick arrow) (Fig. 1G). SLNPs-CIP-treated chicks exhibited minimal inflammatory infiltrates with limited fibroblast proliferation (Fig. 1H), while SLNPs-MEM-treated animals showed perivascular hepatocellular swelling and necrosis (thick arrow) accompanied by mild inflammation (thin arrow) (Fig. 1I).

Photomicrographs of intestinal sections are shown in Figure 2. The control group displayed intact intestinal histoarchitecture, including well-developed mucosal layers and villi (Fig. 2A). In contrast, the infected group exhibited marked pathological lesions such as villous shortening, fusion, and stunting, with extensive lamina propria expansion by inflammatory infiltrates (thin arrow) and hypertrophy of the muscularis layer (arrowhead) (Fig. 2B). Higher magnification revealed pronounced leukocytic infiltration and widely separated crypts (thin arrow) (Fig. 2C, 2D).

MEM-treated chicks displayed mild edema and limited inflammatory cell infiltration within the lamina propria (thin arrow) (Fig. 2E), while CIP-treated birds showed crypt abscesses extending into the muscular layer (thin arrow) (Fig. 2F). In the SLNPs-treated group, moderate to severe thickening of the intestinal wall was observed, with lamina propria expansion due to extensive inflammatory cell infiltration (thin arrow) (Fig. 2G). Chicks treated with SLNPs-CIP exhibited focal apical epithelial sloughing (thick arrow) and villous thickening (thin arrow) (Fig. 2H), whereas SLNPs-MEM-treated intestines showed apical villous thickening and mild lamina propria expansion with a modest number of inflammatory cells (thin arrow) (Fig. 2I).

In general, histological sections confirmed the protective effect of SLNPs-CIP, with minimal inflammatory infiltration and preserved tissue architecture in liver and intestinal tissues, unlike the extensive damage observed in untreated infected groups.

Discussion

The current study endeavoured to establish a new nanotechnology-based therapeutic strategy to struggle with pseudomoniasis disease induced by *P. aeruginosa* in broiler chicks and it is was obvious that oral administration of SLNPs loaded with both CIP and MEM as nano-drug delivery systems demonstrated a marked improvement in the health statue of the infected chicks. This enhancement was corroborated by clinical observations and by both biochemical and gene expression analyses of key immune-modulatory (TNF-α) and antioxidant (SOD) biomarkers. Eraky *et al*. [22] reported that infection of chickens with *P. aeruginosa* induced several clinical symptoms including respiratory signs, reluctance, depression, off food, sleepy appearance, and greenish diarrhoea. The clinical signs vary with the infection dose.

Growth performance is a critical parameter for evaluating the efficacy of novel nano-drug delivery systems in poultry production. Chickens reared in contaminated environments frequently exhibit impaired growth due to intestinal damage and dysfunction caused by bacterial infections [23, 24].

In this context, nanoparticles have garnered attention for their potential to enhance growth performance, feed efficiency, and overall health status in animals [25, 26]. Notably, bacterial pathogens are less likely to develop resistance against the mechanical disruption induced by nanostructured surfaces or the synergistic antimicrobial action of antibiotics delivered *via* controlled nanoemulsion systems [27].

Nano-formulations of essential micro- and macroelements have been consistently shown to promote body weight gain, improve average daily gain (ADG), and enhance feed conversion ratio (FCR) in livestock species [٢٨]. These nanomaterials fulfill nutritional requirements while simultaneously modulating gut microbiota composition, supporting immune responses, and decreasing susceptibility to infections [٢9]. The observed improvements in growth metrics are largely attributed to the unique physicochemical properties of nanomaterials, which facilitate enhanced nutrient absorption by increasing intestinal villus height and width, mucosal thickness, and crypt depth. Consequently, the improved bioavailability of amino acids, calcium, and phosphorus leads to more efficient nutrient utilization and superior growth performance in broilers [30].

Furthermore, the nanoscale particle size of these formulations significantly improves drug solubility and bioavailability, thereby enhancing their interaction with microbial membranes in the gastrointestinal tract. The targeted and sustained release of antibiotic-loaded nanomaterials extends antimicrobial and antioxidant activities, promoting more rapid recovery while concurrently reducing the risk of antimicrobial resistance [31].

The findings of the present study are consistent with those of Sabry *et al*. [32], who demonstrated that treatment of *Salmonella enteritidis*-infected chicks with a nano-drug delivery system specifically, ciprofloxacin-loaded chitosan nanoparticles resulted in significant improvements in both clinical outcomes and body weight gain in treated birds.

Bacterial infections in poultry typically disrupt the redox balance by accelerating the production of free radicals while overwhelming the antioxidant defense systems, a condition known as oxidative stress. The resultant accumulation of ROS leads to cellular damage and inflammation [33]. Measuring the activity of antioxidant enzymes particularly SOD is essential during infection, given its role in dismutating superoxide radicals into less harmful species such as molecular oxygen and hydrogen peroxide [34].

Furthermore, in this non-physiological state, host cells secrete inflammatory cytokines and chemokines and promote the production of endogenous antioxidants which contribute to attracting other cells to fight infection and counteract the adverse effects of these ROS, promoting tissue regeneration. In this non-physiological state, host cells upregulate inflammatory cytokines and chemokines while also promoting endogenous antioxidant mechanisms to counteract oxidative stress and support tissue regeneration [35, 36]. Nanoparticles, due to their high surface area-to-volume ratio, are particularly effective in modulating these responses. They can inhibit key pro-inflammatory mediators such as cytokines and enzymes involved in inflammation [37]. Specifically, nanomaterials have been shown to suppress the nuclear translocation of nuclear factor kappa B (NF-κB) and p65 (an NF-κB family protein) by preventing the cytoplasmic degradation of IκBα, thereby down-regulating the expression of TNF-α [38]. Additionally, nanomaterials can scavenge or neutralize ROS, prevent their synthesis, and stabilize transition metals involved in ROS generation, thereby supporting antioxidant defense [39]. These mechanisms, known as endogenous antioxidant indicators, include the body's own enzymatic and non-enzymatic antioxidant defence mechanisms, such as superoxide dismutase (SOD) representing a primary line of defense against oxidative stress [40].

According to Abdel-Tawwab *et al.* [41] and Hoseinifar *et al*. [42], the production of SOD considers the first defence line against oxidative damage as it reduces or neutralizes the produced reactive oxygen species (ROS) when a host is exposed to a specific stressor. Supplementation of nanomaterials can also reduce oxidative stress and improve serum oxidant status [29].

These results are consistent with those obtained by Elsayed *et al.* [43] who revealed that treatment of tumor-bearing mice with rosemary-loaded solid lipid nanoparticles at two tested doses (200 and 400 mg kg^{-1}) significantly reduced the level of *TNF-α* as compared to the untreated group and concluded that SLNPs can enhance the therapeutic effect of rosemary and improve its efficacy by allowing efficient uptake and release of ROS in a regulated manner. Furthermore, the results obtained Eskandani *et al*. [44] demonstrated that supplementation of low level of L-carnitine-loaded solid lipid nanoparticles (50 mg kg^{-1}) to broiler diet significantly enhanced erythrocyte SOD activity which could be attributed to the high bioavailability of L-carnitine-loaded solid lipid nanoparticles.

TNF-α is a pleiotropic cytokine crucial for host defense against intracellular pathogens, including *Pseudomonas aeruginosa, Mycobacterium tuberculosi*s, and *Salmonella typhimurium*. However, in chickens, the TNF-α gene was difficult to identify due to its GC-rich content and the lack of conserved syntenic regions [45]. The observed gene expression changes may also reflect the modulation of oxidative stress response pathways, particularly the up-regulation of hepatic SOD, consistent with the findings of Shabana *et al*. [46]. Dys-regulation of these genes can contribute to genomic instability, uncontrolled cellular proliferation, and damage to cellular membranes [47].

In this regard, Kishawy *et al*. [48] reported that dietary supplementation with resveratrol-loaded liposomal nanoparticles led to down-regulation of TNF-α expression and up-regulation of antioxidant-related genes, particularly SOD, in both muscle and intestinal tissues of broilers.
enhancing host defense mechanisms and promoting systemic health in broiler chickens afflicted by pseudomoniasis. Future studies should focus on detailed pharmacokinetics and long-term safety.

The small intestine plays a pivotal role in nutrient absorption, and the villus height to crypt depth ratio serves as a key indicator of intestinal health [49]. Although nanoparticle administration has been associated with histological alterations in multiple organs including the liver, pancreas, kidney, intestine, adrenal glands, and brain [50]. The present findings indicate that antibiotic-loaded SLNPs can mitigate infection-induced tissue damage. In support of this, Hosseini *et al*. [6] reported that treatment of Brucella-infected mice with doxycycline-encapsulated SLNPs significantly reduced bacterial load and improved splenic and hepatic histopathology compared to free drug. Similarly, Eskandani *et al*. [44] observed enhanced intestinal absorptive capacity without adverse tissue effects following administration of L-carnitine-loaded SLNPs. Additionally, Sabry *et al* [32] demonstrated notable improvement in hepatic and intestinal histoarchitecture in chicks infected with *Salmonella enteritidis* and treated with ciprofloxacin-loaded chitosan nanoparticles.

Taken together, the observed histological improvements in the liver and intestine coupled with the absence of pathological lesions in SLNPs-treated groups support the efficacy and safety of nanodrug delivery systems, particularly those loaded with ciprofloxacin or meropenem, in combating *Pseudomonas aeruginosa* infections in broiler chickens.

Conclusion

Collectively, these findings support the hypothesis that SLNPs offer a promising strategy for combating antibiotic-resistant infections in poultry by improving drug bioavailability and modulating immune and antioxidant gene expression, thereby

The novel nano-drug delivery systems, SLNPs-CIP and SLNPs-MEM, significantly enhanced the therapeutic efficacy of ciprofloxacin and meropenem, respectively. These nanoformulations achieved approximately 79.0% and 75.8% recovery from illness, compared to 65.7% and 59.9% recovery in groups treated with the free antibiotics. This enhanced therapeutic effect is attributed to the controlled release of the antibiotics within the body, which prolonged their bioavailability and optimized their pharmacodynamic activity. Molecular, biochemical, and histopathological analyses further validated the immunostimulatory potential of these nanodrug systems, demonstrating their effectiveness in mitigating the pathogenic impact of *Pseudomonas aeruginosa* infection in broiler chicks. These findings underscore the potential of SLNP-based formulations as a promising strategy for improving antimicrobial therapy in veterinary medicine.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study follows the

TABLE 1. Nutrient composition of the basal diet.

Ingredients	Content (%)	Chemical composition	Content
Yellow corn	62.13	Metabolizable Energy (Kcal kg $^{-1}$)	3160
Soybean meal	28.12	Crude protein (%)	20.86
Corn gluten meal (60%)	4.00	Methionine (%)	0.60
Soybean oil	1.80	Lysine (%)	1.35
Limestone	1.15	Methionine+ Cysteine (%)	1.00
Dicalcium phosphate	1.45	Calcium (%)	0.94
Vitamin and mineral mixture [a]	0.30	Nonphytate P (%)	0.45
Salt	0.25		
DL-Methionine	0.25		
L-lysine HCl	0.20		
Choline chloride	0.10		
Sodium bicarbonate	0.25		

Level of nutrient in the diet was based on **NRC (1994).**
[a] Vitamin-mineral mixture supplied per kilogram of diet: Vit A: 15000 IU, Vit D_3: 2000 IU, Vit E: 20 mg, Vit K_3 5 mg, Vit B_2: 5 mg, Vit B_1: 2 mg, Vit B_{12}: 0.02 mg, Vit B_6: 2 mg, Pantothenic acid: 12 mg, Biotin: 0.1 mg, Niacin, 25 mg, Folic acid: 1 mg, Copper: 5 mg, Iodine: 1 mg, Manganese: 70 mg, Iron: 50 mg, Zinc: 50 mg and Selenium: 0.1 mg.

TABLE 2. Real-time PCR primers made of oligonucleotides that are forward and reverse for investigated genes under study.

Investigated marker	Primer	Product size (bp)	Annealing Temperature (°C)	GenBank isolate
TNF-α	F5′- CACACTTCGGGCAGCTCTTA -3 R5′- AGGGTTATTTCAGCCCCGTG -3′	133	60	NM_001024578.2
SOD	F5′- ACCCCTTTGGAGTGAACCAC -3 R5′- TGGATCACAACGGATCTGCC -3′	145	60	XM_040699307.2
ß. actin	F5′- TGAATCCGGACCCTCCATTG -3′ R5′- AGACTGCTGCTGACACCTTC -3′	195	58	L08165.1

TABLE 3. External clinical symptoms of pseudomonasis disease caused by *Pseudomonas aeruginosa* in all broiler chicks groups at 24 d-old (Before treatment).

External symptoms	Appearance	% change
Weakness	+++	100
Diarrhea	++	٧٥
Localized external lesions in different organs	+	18.1
Loss of appetite	+	24.2
Depression	+	15.1
Closed eye	+	12
Sinusitis	Nill	--
Respiratory manifestations	++	٨٤.9
Eratitis	Nill	--
Keratoconjunctivitis	+	6.06

- (+++; Severe symptom, ++; Strong symptom, +; Mild symptom)
- % Change was calculated as: (Number of infected chicks / Total number of chicks in the group) X 100

TABLE 4. Effect of ciprofloxacin, meropenem and SLNPs either singly or in combination as nanodrug delivery systems on the body weight of broiler chicks diseased by pseudomoniasis disease throughout the entire period of experiment.

Parameter / Treatment	Initial wt. (7-d –old)	Starting infection (14 -d -old)	Starting recovery (24-d- old)	Sampling (36-d- old)	%Recovery As control (infected)
Healthy	$550^{j} \pm 4.05$	968^{h}	1680^{e}	2853^{a}	----
infected	$546^{j} \pm 4.3$	885^{i}	1369^{g}	1534^{f}	----
CIP	$542^{j} \pm 4.05$	930^{h}	1584^{f}	2543^{c}	65.7
MEM	$545^{j} \pm 4.1$	914^{i}	1539^{f}	2453^{d}	59.9
SLNPs	$542^{j} \pm 4.09$	932^{h}	1573^{f}	2637^{c}	71.9
SLNPs- CIP	$552^{j} \pm 4.12$	943^{h}	1583^{f}	2746^{b}	79.0
SLNPs-MEM	$543^{j} \pm 4.02$	956^{h}	1587^{f}	2698^{c}	75.8

Mean values listed are given as gm per chick ± standard error with different superscript letter (a, b, c, d, e, f, g, h, i, j) in the same column are significantly $p \leq 0.05$.

% Recovery was calculated as: (Mean value of treated chicks- mean value of infected chicks/ mean value of infected chicks) X 100

TABLE 5. Effect of ciprofloxacin, meropenem and SLNPs either singly or in combination as nanodrug delivery systems on hepatic and intestinal TNF-α concentration of broiler chicks diseased by pseudomoniasis disease. Means of within the same column having different upper-case superscripts are significantly different at $p \leq 0.05$.

Parameter / Treatment	TNF-α (Pg/mg protein)		% Change		% Recovery	
	Liver	Ileum	Liver	Ileum	Liver	Ileum
Control; Healthy	135.2^{m}	104.6^{m}	-	-	--	--
Control; Infected	316.9^{a}	283.1^{a}	134.3	170.6	--	--
CIP	249.2^{f}	215.0^{f}	84.3	105.5	-21.3	-24.05
MEM	261.7^{e}	232.1^{e}	93.56	121.8	-17.4	-18.01
SLNPs	281.5^{c}	252.8^{c}	108.2	141.6	-11.1	-10.7
SLNPs-CIP	151.0^{k}	135.2^{k}	11.6	29.2	-52.3	-52.2
SLNPs-MEM	185.0^{i}	160.8^{i}	36.8	53.7	-41.6	-43.2

- % Change was calculated as: (Mean value of treated chicks- mean value of healthy chicks/ mean value of healthy chicks) X 100
- % Recovery was calculated as: (Mean value of treated chicks- mean value of infected chicks/ mean value of infected chicks) X 100

TABLE 6. Effect of ciprofloxacin, meropenem and SLNPs either singly or in combination as nanodrug delivery systems on hepatic and intestinal SOD activity of broiler chickens diseased by pseudomoniasis disease. Means of within the same column having different upper-case superscripts are significantly different at p ≤ 0.05.

Parameter / Treatment	SOD (Pg/mg protein)		% Change		% Recovery	
	Liver	Ileum	Liver	Ileum	Liver	Ileum
Control; Healthy	216.4^{a}	178.3^{a}	-	-	--	--
Control; Infected	68.2^{M}	48.1^{M}	-68.4	-73.02	--	--
CIP	118.2^{g}	94.1^{g}	-45.3	-47.2	73.3	95.6
MEM	75.7^{L}	59.0^{L}	-68.4	-66.9	10.9	176.2
SLNPs	93.6^{j}	72.7^{j}	-56.7	-59.2	37.2	51.1
SLNPs-CIP	189.9^{c}	139.4^{c}	-12.2	-21.8	178.4	189.8
SLNPs-MEM	105.5^{i}	81.0^{i}	-51.2	-54.5	54.6	68.3

- % Change was calculated as: (Mean value of treated chicks- mean value of healthy chicks/ mean value of healthy chicks) X 100
- % Recovery was calculated as: (Mean value of treated chicks- mean value of infected chicks/ mean value of infected chicks) X 100

TABLE 7. Effect of ciprofloxacin, meropenem and SLNPs either singly or in combination as nanodrug delivery systems on gene expression profile of hepatic and intestinal TNF-α of broiler chicks diseased by pseudomoniasis disease. Means of within the same column having different upper-case superscripts are significantly different at p ≤ 0.05.

Parameter / Treatment	TNF-α (2^−ΔΔCt)		% Change		% Recovery	
	Liver	Ileum	liver	ileum	liver	ileum
Control; Healthy	$0.489+0.16^{i}$	$0.351+0.21^{j}$	--	--	--	--
Control; Infected	$3.451+0.21^{a}$	$2.841+0.14^{a}$	605.7	709.4	--	--
CIP	2.154+0.18d	1.841+0.14e	340.4	424.5	-37.5	-35.1
MEM	$2.051+0.09^{d}$	$1.781+0.25^{e}$	319.4	407.4	-40.5	-37.3
SLNPs	$2.451+0.08^{c}$	$2.051+0.11^{cd}$	401.2	484.3	-28.9	-27.8
SLNPs-CIP	$0.845+0.514^{h}$	$0.451+0.18^{j}$	72.8	28.4	-75.5	-84.1
SLNPs-MEM	$1.284+0.14^{g}$	$1.084+0.05^{h}$	162.5	208.8	-62.7	-61.8

- % Change was calculated as: (Mean value of treated chicks- mean value of healthy chicks/ mean value of healthy chicks) X 100
- % Recovery was calculated as: (Mean value of treated chicks- mean value of infected chicks/ mean value of infected chicks) X 100

TABLE 8. Effect of ciprofloxacin, meropenem, and SLNPs either singly or in combination as nanodrug delivery systems on gene expression profile of hepatic and intestinal SOD of broiler chicks diseased by pseudomoniasis disease. Means of within the same row having different upper-case superscripts are significantly different at p ≤ 0.05.

Parameter / Treatment	SOD (2^−ΔΔCt)		% Change		% Recovery	
	Liver	Ileum	liver	ileum	liver	ileum
Control; Healthy	$3.184+0.19^{a}$	$2.548+0.28^{a}$	--	--	--	--
Control; Infected	$0.863+0.43^{i}$	$0.369+0.22^{j}$	-72.8	-85.5	--	--
CIP	$1.694+0.08^{ef}$	$1.215+0.18^{f}$	-46.7	-52.3	96.2	229.2
MEM	$1.584+0.19^{f}$	$1.084+0.36^{fg}$	-50.2	-57.4	83.5	194.5
SLNPs	$1.289+0.06^{j}$	$0.984+0.15^{h}$	-59.5	-61.3	49.3	166.6
SLNPs-CIP	$2.486+0.28^{c}$	$2.145+0.45^{c}$	-21.9	-15.18	188	481.3
SLNPs-MEM	$2.169+0.24^{d}$	$1.698+0.29^{d}$	-31.8	-33.3	151.3	360.1

- % Change was calculated as: (Mean value of treated chicks- mean value of healthy chicks/ mean value of healthy chicks) X 100
- % Recovery was calculated as: (Mean value of treated chicks- mean value of infected chicks/ mean value of infected chicks) X 100

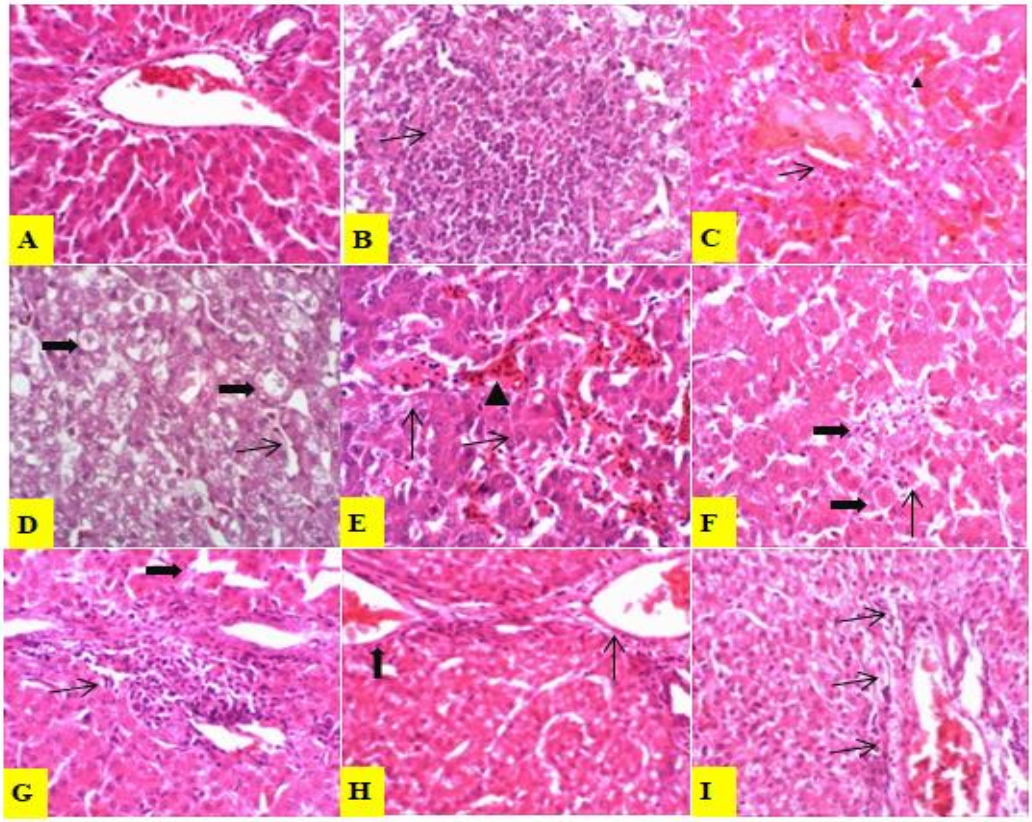

Fig. 1. Representative photomicrograph of hepatic section of A; healthy chicks, B, C, D; infected chicks, E; chicks treated with MEM, F; chicks treated with CIP, G; chicks treated with SLNPs, H; chicks treated with SLNPs-CIP, I; chicks treated with SLNPs-MEM.

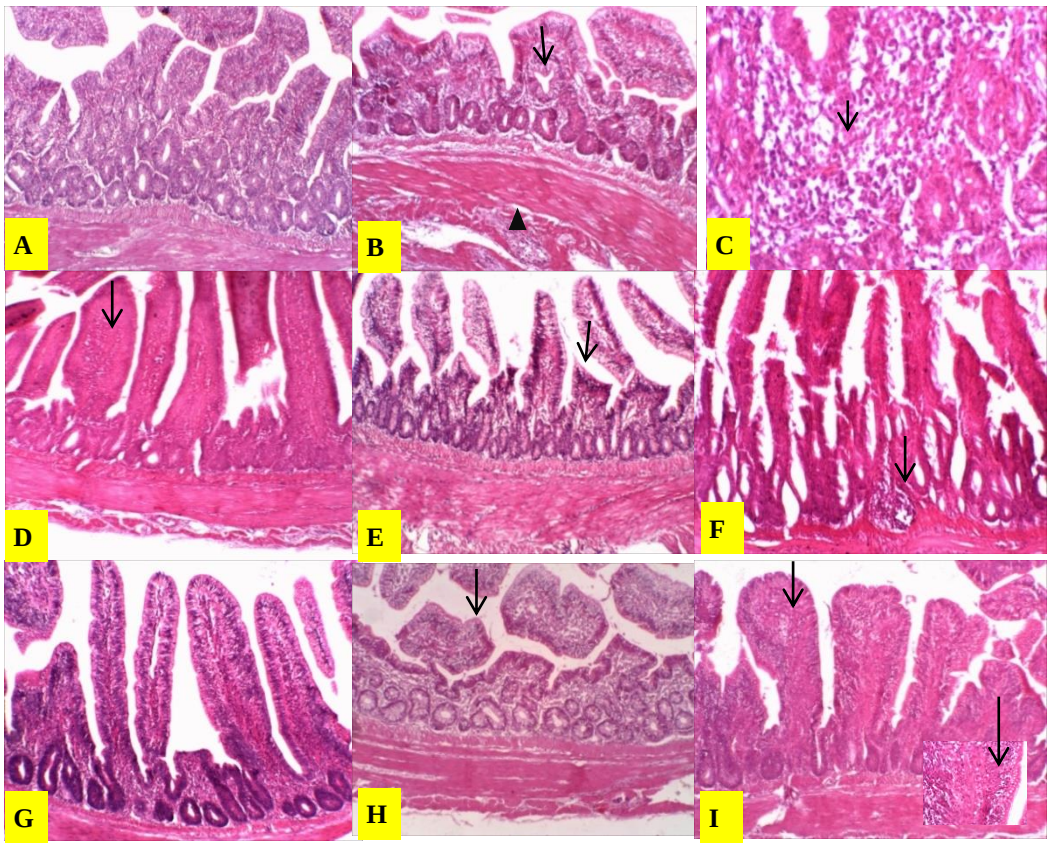

Fig. 2. Representative photomicrograph of intestinal section of A; healthy chicks, B, C, D; infected chicks, E; chicks treated with MEM, F; chicks treated with CIP, G; chicks treated with SLNPs, H; chicks treated with SLNPs-CIP, I; chicks treated with SLNPs-MEM.

References

1. Hosseini, M., Taheri, M., Nouri, F.A., Farmani, N. M., Moez, M. and Arabestani, R. Nanodrug delivery in intracellular bacterial infection treatments. *Biomedicine & Pharmacotherapy*. **146,** 112609 (2022). DOI: 10.1016/j.biopha.2021.112609
2. Ali, A. and Ahmed S. A review on chitosan and its nanocomposites in drug delivery. *International Journal of Biological Macromolecules*, **109,** 273-286 (2018). DOI: 10.1016/j.ijbiomac.2017.12.078
3. Müller, R., Maaβen, S. Weyhers, H., Specht, F. and Lucks, J. Cytotoxicity of magnetite-loaded polylactide, polylactide/glycolide particles and solid lipid nanoparticles. *International Journal of Pharmaceutics*, **138** (1), 85-94 (1996). DOI: 10.1016/0378-5173(96)04539-5.
4. Helal, S.H., Abdel-Aziz, H.M.M., El-Zayat, M.M. and Hasaneen, M.N.A. Preparation, characterization and properties of three different nanomaterials either singly or loaded with nystatin or fluconazole antifungals. *Scientific Reports*, **12** (1), 22110 (2022). DOI: 10.1038/s41598-022-26523-1
5. George, D., Lakshmi, S., Sharma, A., Prakash, S., Siddiqui, M., Malavika, B.R. and Elumalai, P. Nanotechnology: A novel tool for aquaculture feed development. In: *Nanotechnological approaches to the advancement of innovations in aquaculture* (eds. Kirthi, A.V., Loganathan, K. & Karunasagar, I.). *Nanotechnology in the Life Sciences*, Springer, Cham. pp. 67-88 (2023). DOI: 10.1007/978-3-031-15519-2_4
6. Hosseini, S.M., Farman, A., Abbasalipourkabir, R., Asl, S., Nourian, S.A. and Arabestani, M.R. Doxycycline-encapsulated solid lipid nanoparticles for the enhanced antibacterial potential to create the chronic brucellosis and preventing its relapse: *in-vivo* Study. *Annals of Clinical Microbiology and Antimicrobial*, **18**, 33 (2019). DOI: 10.1186/s12941-019-0333-x .
7. Mohamed, H.A.E.H. Some studies on *Pseudomonas* species in chicken embryos and broilers in Assiut governorate. *Assiut University Bulletin Environmental Researches*, **7**(1), 23-30 (2004). DOI:10.21608/auber.2004.150590
8. Mena, K.D. and Gerba, C.P. Risk Assessment of *Pseudomonas aeruginosa* in water. *Reviews of Environmental Contamination and Toxicology*, **201**, 71-115 (2009). DOI:10.1007/978-1-4419-0032-6_3
9. John-Barnes, H. Other bacterial diseaes: *Pseudomonas*. In: *Diseases of poultry*, (eds. Calnek, B. W., John Barnes, H., Beard, C.W., Mcdougald L.R. & Saif, Y. M.) 10th edition P. 291 – 292. (1997).
10. Hussein, S.A., Hassan, A.H. and Sulaiman, R.R. Bacteriological and pathological study of yolk sac infection in broiler chicks in sulaimani district. *Journal of Dohuk University*, **11**(1),48-56 (2008).
11. Hai-ping, H.E. Isolation and identify of *Pseudomonas aeruginosa* in chicken dead-embryos. *Chinese Qinghai Journal of Animal Veterinary Sciences*, **3**, 25-27 (2009).
12. Atta, A.H., and Sharif, L. Pharmacokinetics of ciprofloxacin following intravenous and oral administration in broiler chickens. *Journal of Veterinary Pharmacology and Therapeutics*, **20** (4), 326-329 (1997). DOI:10.1046/j.1365-2885.1997.00065.x
13. Doi, Y. Treatment options for carbapenem-resistant gram-negative bacterial infections. *Clinical Infectious Disease*, **69**, 565–575 (2019). DOI: org/10.1093/cid/ciz830
14. NRC (National Research Council). Nutrient requirements of poultry. (9th rev. Ed.) National Academy Press. Washington, D.C., USA pp. 26-34 (1994). DOI:10.17226/2114
15. Ateya, A.I., Arafat, N., Saleh, R.M., Ghanem, H.M., Naguib, D., Radwan, H.A. and Elseady, Y.Y. Intestinal gene expressions in broiler chickens infected with *Escherichia coli* and dietary supplemented with probiotic, acidifier and symbiotic. *Veterinary Research Communications*, **43**, 131–142 (2019). DOI:10.1007/s11259-019-09753-z
16. Shoumali, L., Masoud, H., Khlaif, H., Migdadi, H. and Masoud, S. Serologic and molecular characterization of *Pseudomonas aeruginosa* Jordanian clinical isolates compared with the strains of International Antigenic Typing Scheme. *Diagnostic Microbiology and Infectious Diseases*, **58** (4), 393-8 (2007). DOI:10.1016/j.diagmicrobio.2007.03.005
17. Payte, G.S., Purnamasari, L., Olarve, J.P., de Leon, N. J. and Pdela Cruz, J.F. Correlation between body weight day old chick (doc) and body weight each week from commercial farms in province of rizal, philippines. *Jurnal Ilmu Produksi dan Teknologi Hasil Peternakan*, **10** (3), 126-131(2022). DOI:10.29244/jipthp.10.3.126-131
18. Al-Khatib, B.Y., Al-Hamdani, N.M. and Gumaih, H.S. Ameliorated and antioxidant effects of fucoidan against cyclosporine A-induced kidney injury in rats. *Journal of Basic and Applied Zoology*, **80** (1), 11 (2019). DOI:10.1186/s41936-019-0108-0
19. Mousa, S., Elsayed, A., Marghani, B. and Ateya, A. Effects of supplementation of *Bacillus* spp. on blood metabolites, antioxidant status, and gene expression pattern of selective cytokines in growing Barki lambs. *Journal of Advanced Veterinary and Animal Research*, **6**, 333-340 (2019). DOI: org/10.5455/javar.2019.f351
20. Pfaffl, M.W. A new mathematical model for relative quantification in real-time RT–PCR. *Nucleic Acids Research*, **29** (9), e45-e45 (2001). DOI:10.1093/nar/29.9.e45
21. Bancroft, J.D. and Gamble, M. Theory and Practice of histological techniques, *Elsevier Health Sciences*,50006-3 (2008). DOI:10.1016/b978-0-443-10279-0.50006-3
22. Eraky, R.D., Abd EL-ghany, W.A. and Soliman, K.M. Studies on *Pseudomonas aeruginosa* infection in hatcheries and chicken. *Journal of the Hellenic Veterinary Medical Society*, **71**(1), 1953–1962 (2020). DOI:10.12681/jhvms.22937

23. Cao, G.T., Zeng, X.F., Chen, A.G., Zhou, L., Zhang, L., Xiao, Y.P. and Yang, C. Effects of a probiotic, *Enterococcus faecium*, on growth performance, intestinal morphology, immune response, and cecal microflora in broiler chickens challenged with Escherichia coli K88. *Poultry Science*, **92**, 2949–2955 (2013). DOI:10.3382/ps.2013-03366

24. Gao, Y., Han, F., Huang, X., Rong, Y., Yi, H. and Wang, Y. Changes in gut microbial populations, intestinal morphology, expression of tight junction proteins, and cytokine production between two pig breeds after challenge with *Escherichia coli* K88: a comparative study. *Journal of Animal Science*, **91**, 5614–5625 (2013). DOI:10.2527/jas.2013-6528

25. Amlan, P. and Lalhriatpuii, M. Progress and prospect of essential mineral nanoparticles in poultry nutrition and feeding – a review. *Biological Trace Element Research*, **197**(1), 233–253 (2020). DOI:10.1007/s12011-019-01959-1

26. Dawood, M.A.O., Basuini, M.F.E., Yilmaz, S., Abdel-Latif, H.M.R., Kari, Z.A., Abdul Razab, M.K.A., Ahmed, H.A., Alagawany, M. and Gewaily, M.S. Selenium nanoparticles as a natural antioxidant and metabolic regulator in aquaculture: a review. *Antioxidants*, **10** (9), 1364 (2021). DOI:10.3390/antiox10091364

27. Tripathy, A., Sen, P., Su, B. and Briscoe, W.H. Natural and bioinspired nanostructured bactericidal surfaces. *Advances in Colloid Interface Science*, **248**, 85–104 (2017). DOI:10.1016/j.cis.2017.07.030

28. Yusof, H.M., Mohamad, R., Zaidan, U.H. and Rahman, N.A.A. Microbial synthesis of zinc oxide nanoparticles and their potential application as an antimicrobial agent and a feed supplement in animal industry: a review. *Journal of Animal Science and Biotechnology*, **10** (57), (2019). DOI:10.1186/s40104-019-0368-z

29. Michalak, I., Dziergowska, K., Alagawany, M., Farag, M. R., El-Shall, N. A., Tuli, H. S. and Dhama, K. The effect of metal-containing nanoparticles on the health, performance and production of livestock animals and poultry. *Veterinary Quarterly*, **42**(1), 68–94 (2022). DOI: 10.1080/01652176.2022.2073399

30. Ali, N.M., Mohamed, G.A.E. and El-Demerdash, A.S. Impact of oral administration of chitosan-nanoparticles on oxidative stress index and gut microbiota of heat stressed broilers. *Journal of Advanced Veterinary Research*, **13** (6), 997-1003 (2023).

31. Movahedi, F., Nirmal, N., Wang, P., Jin, H., Grøndahl, L. and Li, L. Recent advances in essential oils and their nanoformulations for poultry feed. *Journal of Animal Science and Biotechnology*, **15**, 110 (2024) . DOI:10.1186/s40104-024-01067-8

32. Sabry, S.M., Omer, A.M., El-Sayed, A.K.A., Ateya, A.I. and Hasaneen, M.N.A. A novel *in-vivo* drug delivery strategy against *Salmonella*: A case study of ciprofloxacin loaded chitosan nanoparticles for broiler birds. *Mansoura Journal of Biology*, **71**, 1-16 (2024).

33. Lee, M.T., Lin, W.C., Yu, B. and Lee, T.T. Antioxidant capacity of phytochemicals and their potential effects on oxidative status in animals—a review. *Asian-Australasian Journal of Animal Science*, **30**, 299–308 (2016). DOI:10.5713/ajas.16.0438

34. Zeng, T., Li, J.J., Wang, D.Q., Li, G.Q., Wang, G.L. and Lu, L.Z. Effects of heat stress on antioxidant defense system, inflammatory injury, and heat shock proteins of Muscovy and Pekin ducks: Evidence for differential thermal sensitivities. *Cell Stress Chaperones*, **19** (6), 895–901 (2014). DOI:10.1007/s12192-014-0514-7

35. Laskin, D. L., Sunil, V. R., Gardner, C. R. and Laskin, J. D. Macrophages and tissue injury: agents of defense or destruction?. *Annual Review of Pharmacology and Toxicology*, **51**, 267–288 (2011). DOI:10.1146/annurev.pharmtox.010909.105812

36. Shahidi, F. Handbook of antioxidants for food preservation. Woodhead Publishing Series in Food Science, Technology and Nutrition (2015). DOI:10.1016/b978-1-78242-089-7.09002-3

37. Agarwal, H., Nakara, A. and Shanmugam, V.K. Anti-inflammatory mechanism of various metal and metal oxide nanoparticles synthesized using plant extracts: A review. *Biomedicine & Pharmacotherapy*, **109**, 2561–2572 (2019). DOI:10.1016/j.biopha.2018.11.116

38. Kim, M.H. and Jeong, H.J. Zinc oxide nanoparticles suppress LPS-induced NF- κ B activation by inducing A20, a negative regulator of NF- κ B, in RAW 264.7 Macrophages. *Journal of Nanoscience and Nanotechnology*, **15**, 6509–6515 (2015). DOI:10.1166/jnn.2015.10319

39. Masella, R., Benedetto, R.D., Varı, R., Filesi, C. and Giovannini, C. Novel mechanisms of natural antioxidant compounds in biological systems: involvement of glutathione and glutathione-related enzymes. *Journal of Nutritional Biochemistry*, **16**, 577 – 586 (2005). DOI:10.1016/j.jnutbio.2005.05.013

40. Glasauer, A. and Chandel, N.S. Targeting antioxidants for cancer therapy. *Biochemical Pharmacology*, **92** (1), 90-101 (2014). DOI:10.1016/j.bcp.2014.07.017

41. Abdel-Tawwab, M., Khattaby, A.-R.A. and Monier, M.N. Dietary acidifiers blend enhanced the production of Nile tilapia (*Oreochromis niloticus*), striped mullet (*Mugil cephalus*), and African catfish (*Clarias gariepinus*) polycultured in earthen ponds. *Aquaculture International*, **27** (2), 369–379 (2018). DOI:10.1007/s10499-018-0329-0

42. Hoseinifar, S. H., Yousefi, S., Van Doan, H., Ashouri, G., Gioacchini, G., Maradonna, F. And Carnevali, O. Oxidative Stress and Antioxidant Defense in Fish: The Implications of Probiotic, Prebiotic, and Synbiotics. *Reviews in Fisheries Science and Aquaculture*, **29** (2), 198–217 (2020). DOI:10.1080/23308249.2020.1795616

43. Elsayed, D.H., Said, N.I., Said, A.A., Hamad, H.R. and Abd-Elrazek, A.M. Anticarcinogenic activity of rosemary loaded solid lipid nanoparticle against solid tumor in mice. *Labyrinth: Fayoum Journal of Science and Interdisciplinary*, **2** (1), 8-13 (2024). DOI:10.21608/ifjsis.2024.263353.1056

44. Eskandani, R., Kazempour, M., Farahzadi, R., Sanaat, Z., Eskandani, M., Adibkia, K., Vandghanooni, S. and Ahad, M. Engineered nanoparticles as emerging

gene/drug delivery systems targeting the nuclear factor-κB protein and related signaling pathways in cancer. *Biomedicine and Pharmacotherapy*, **156**, 113932 (2022). DOI:10.1016/j.biopha.2022.113932

45. Rohde, F., Schusser, B., Hron, T., Farkašová, H., Plachý, J., Härtle, S., Hejnar, J, Elleder, D. and Kaspers, B. Characterization of chicken tumor necrosis factor-α, a long missed cytokine in birds. *Frontiers in Immunology*,**9**,605(2018). DOI:10.3389/fimmu.2018.00605

46. Shabana, S. M., Elbaz, S., Abass, M.E. and Abu-zahra, N.I.S. Effects of dietary sodium butyrate on the biological indices, gene expression, and resistance of oreochromis niloticus to multidrug-resistant *Pseudomonas aeruginosa* infection, *Assiut Veterinary Medical Journal*, **183** (70), 335-360 (2024). DOI:10.21608/avmj.2024.301270.1291

47. Kaur, H., Kaur, K., Singh, A., Bedi, N., Singh, B., Alturki, M.S., Aldawsari, M.F., Almalki, A.H., Haque, S., Lee, H-J, Yadav, D.K. and Arora, S. Frankincense oil-loaded nanoemulsion formulation of paclitaxel and erucin: A synergistic combination for ameliorating drug resistance in breast cancer: In vitro and in vivo study. *Frontiers in Pharmacology*, **13**, 1020602 (2022). DOI:10.3389/fphar.2022.1020602

48. Kishawy, A.T.Y., Ibrahim, D., Roushdy, E.M., Moustafa, A., Eldemery, F., Hussein, E. M., Hassan, F. A.M., Elazab, S.T., Elabbasy, M.T., Kanwal, R., Kamel, W.M., Atteya, M.R., and Zaglool, A.W. Impact of resveratrol-loaded liposomal nanocarriers on heat-stressed broiler chickens: effects on performance, sirtuin expression, oxidative stress regulators, and muscle building factors. *Frontiers in Veterinary Science*, **10**, 1137896 (2023). DOI:10.3389/fvets.2023.1137896

49. Biasato, I., Ferrocino, I., Biasibetti, E., Grego, E., Dabbou, S., Sereno, A., Gai, F., Gasco, L., Schiavone, A. and Cocolin, L. Modulation of intestinal microbiota, morphology and mucin composition by dietary insect meal inclusion in free-range chickens. *BMC Veterinary Research*, **14**, 383 (2018). DOI:10.1186/s12917-018-1690-y

50. Bąkowski, M., Kiczorowska, B., Samolińska, W., Klebaniuk, R. and Lipiec, A. Silver and zinc nanoparticles in animal nutrition – a review. *Annals of Animal Science*, **18**(4), 879–898 (2018). DOI:10.2478/aoas-2018-0029

التقييم الحيوي لدقائق الدهن الصلب النانومترية المحمّلة بالسيبروفلوكساسين والميروبينيم كمنصة نانوية واعدة مضادة للميكروبات ضد بكتيريا سيدوموناس ايروجينوزا في الدجاج

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الملخص

تهدف هذه الدراسة إلى تقييم فعالية دقائق الدهن الصلب النانومترية والمعطاه عن طريق الفم سواء منفردة أو محمّلة بالمضادين الحيويين سيبروفلوكساسين وميروبينيم، كنظم جديدة لتوصيل الأدوية النانومترية ضد العدوي التى تسببها بكتيريا الزائفة الزنجارية(*Pseudomonas aeruginosa*) في الدجاج المصاب. تم دراسة تأثير هذه الأنظمة النانومترية على وزن ونمو الدجاج المصاب، والدلالات الكيميائية الحيوية، وتعبير بعض الجينات المرتبطة بالمناعة ومضادات الأكسدة وتحديدا TNF-α وإنزيم السوبرأوكسيد ديسميوتاز (SOD) ومقارنتها بالعلاج التقليدي بالمضادات الحيوية منفردة وأظهرت النتائج أن دقائق الدهن الصلب النانومترية التي يقل حجمها عن ١١٠ نانومتر أدت إلي نتائج علاجية محسّنة بشكل ملحوظ عند تحميلها بسيبروفلوكساسين أو ميروبينيم، حيث حسنت بشكل واضح من أعراض المرض الظاهرية كما أدت هذه الأنظمة إلى زيادة كبيرة في وزن الدجاج المصاب مقارنة بالمجموعات المعالجة بالمضادات الحيوية الحرة (غير محمّلة). كما أوضحت النتائج أن تلك الأنظمة النانومترية أدت إلى إنخفاض كبير في تركيزات (TNF-α) ، بالإضافة إلى إنخفاض التعبير الجيني له، وزيادة ملحوظة في نشاط إنزيم السوبر أوكسيد ديسميوتاز (SOD) مع تعزيز التعبير الجيني له في كل من الكبد والأمعاء وارتبطت هذه التغيرات الجزيئية والكيميائية الحيوية بتحسن نسيجي في أنسجة الكبد والأمعاء. ولقد تم تسجيل نسب الشفاء من المرض مقارنة بالعينات المصابة وكانت كالتالي: ٧١.٩٪ في العينات المعاملة بدقائق االدهن الصلب النانومترية منفردا و ٦٥,٧٪ في العينات المعاملة بالسيبروفلوكساسين منفردا و ٥٩,٩ ٪ في العينات المعاملة بالميروبينيم منفردا و ٧٩٪ في العينات المعاملة بدقائق الدهن الصلب النانومترية المحملة بالسيبروفلوكساسين و٧٥,٨٪ في العينات المعاملة بدقائق الدهن الصلب النانومترية المحملة بالميروبينيم وذلك خلال فترة التجربة. ويمكن الإستنتاج إلى أنه بالمقارنة مع نظام العلاج التقليدي بالسيبروفلوكساسين والميروبينيم فقط، فقد أظهرت النتائج أن دقائق الدهن الصلب النانومترية المحمّلة بسيبروفلوكساسين وميروبينيم تُمثل نظاما نانومتريًا علاجيًا بديلًا وواعدًا للتحكم في عدوى سيدوموناس ايروجينوزا في الدواجن، لما توفره من تحسين في توافر الدواء الحيوي، وزيادة فترة التأثير المضاد للبكتيريا، وتقليل تلف الأنسجة المصابة.

الكلمات الدالة: سيدوموناس ايروجينوزا ، دقائق الدهن الصلب النانومترية، سيبروفوكساسين، ميروبينيم، نظم توصيل الدواء النانومترية.