Archives of Razi Institute, Vol. 77, No. 5 (2022) 1821-1829

DOI: 10.22092/ARI.2022.358493.2233

Original Article

in vivo Evaluation of Immunomodulatory Activity of Lipopolysaccharide Zinc Oxide Nanoparticles (LPS-ZnNPS)

Abdel Razak, F. S[1 *], AlJoofy, I. K[1], Zaki, N. H[1]

1. Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq

Received 27 April 2022; Accepted 26 May 2022
Corresponding Author: fatemasamer1987@gmail.com

Abstract

Klebsiella pneumoniae causes a wide variety of infectious diseases. Lipopolysaccharide (LPS) is a large heat-stable polymer that is gram-negative bacteria's major outer membrane component, accounting for roughly 75% of the surface area and 5-10% of the total dry weight. Therefore the current *in vivo* study was carried out to investigate the immunomodulatory effect of purified lipopolysaccharide produced from local clinical *Klebsiella pneumoniae* isolates compared with ZnO-NPs and LPS-ZnO NPs. To do the experimental evaluations 35, Balb/c mouse was injected intramuscularly (i.m.) with different concentrations of the purified LPS, ZnoNPs and LPS-ZnoNPs for 12 days and immunized with 10% SRBCs (i.p) on day 4 and 8 of the schedule, while *K. pneumoniae* suspension and normal saline for positive and negative control groups. Focus on estimating body weight before and after treatment, Arthus and delayed-type hypersensitivity, and detecting serum level of cytokines (TLR-2, IL1Beta, IL4, and IL10) using sandwich ELISA. The data showed the highest value before and after treatment with LPS-ZnO NPs recorded in 2µg/mouse was 27. 92±1.48 and 31.50±0.4, respectively. In Arthurs reaction and Delayed type hypersensitivity, the highest results showed in the positive control group injected with *K. pneumoniae* 4.08±0.17 and 4.86±80.02, respectively. The results of TLR-2 showed the highest value in the positive control group, 242.17±3.98 pg/ml, followed by Group LPS at 135.51.58 pg/ml. The results of Interleukin-1Beta showed the highest value in the positive control group, 254.88±3.51 pg/ml, followed by Group LPS 174.3± 1.46 pg/ml. The concentration of IL-4 in serum of treated albino mice showed the highest value in the positive control group, 136.2±1.12 pg/ml, followed by Group LPS 86.12±1.49 pg/ml. While the highest value of IL-10 was recorded in the positive control group, 98.58± 4.09 pg/ml, followed by Group LPS-ZnoNP in concentration 4µg/ mouse was 86.018±0.69 pg/ml. The results of the statistical analysis showed a significant difference ($P \leq 0.05$) between LPS, ZnoNPs, and LPs-ZnoNPs treated groups and control groups (positive & negative). In the present study, we can conclude that LPS-ZnO NPs had a positive immunomodulatory effect on immune response in immunized mice. As shown in the results of the level of IL-1 beta, IL-4, IL-10, and TLRs-2, Abs titer, and Arthus and DTH reactions.

Keywords: *K. pneumoniae*, LPS- ZnO NPs, Arthus, DTH Reactions, Cytokines, TLR-2

1. Introduction

Klebsiella pneumoniae causes a wide variety of infectious diseases. It is a Gram-negative, rod shape, non-motile, facultatively anaerobic, lactose fermenter with a protuberant capsule bacillus, which is universally present in the environment such as water, soil, vegetation and freely isolated from mammalian mucosal surfaces (1), incapable of growth at 10ºC, not forming H2S and negative for indole test (2). Lipopolysaccharide (LPS) is a large heat-stable polymer that is gram-negative bacteria's major outer membrane component, accounting for roughly 75% of the surface area and 5-10% of the total dry weight. Lipid A, core oligosaccharide, and repeating polysaccharide designated as ″O″ antigen make up their basic composition. Lipid A is highly conserved and is

responsible for its toxicity due to its endotoxic action (3).

Nano-technology is high-speed science related to other sciences like Biology, Physics, Chemistry, Engineering, and other sciences. It deals with Nanoparticles sized 0.1 to 100 nm; also, these compounds display properties like electrical conductance, chemical reactivity, magnetism, thermal conductivity, chemical stability, physical strength, and optical effects from huge compounds due to their small size (4).

Zinc oxide nanoparticles are inorganic metal oxide that can be used safely as a medication, antibacterial agent, and package preservative. It quickly permeates meals and eliminates microorganisms. Nanoparticles of antibacterial zinc oxide infiltrate the cell membrane and inhibit the development of pathogens. By oxidative stress, lipids, proteins, carbohydrates, proteins, and DNA are all harmed (5).

Cytokines are hormone-like glycoproteins with low-molecular synthesized by different immune cells, mainly by T cells, neutrophils, and macrophages, which are responsible for promoting and regulating immune responses such as activity, differentiation, proliferation, and production of cells. These glycoproteins act on signaling molecules and cells, stimulating them toward sites of inflammation, infections, and traumas and acting on primary lymphocyte growth factors and other biological functions (6).

IL-1 beta is a pro-inflammatory cytokine produced by a limited number of cell types, such as circulating immune cells, monocytes, tissue macrophages, and dendritic cells. It plays a central role in generating and regulating inflammation as a part of the immune response during inflammation and in influencing additional physiological and pathological functions such as auto-immune diseases and malignancies (7).

IL-4 is a cytokine produced by Th2 cells, mast cells, basophils, eosinophils, and neutrophils that stimulates a diverse array of biological responses. It plays a vital role in regulating inflammation and immune responses. Interleukin-4 plays a critical regulatory role in allergic responses and has antitumor and anti-inflammatory effects. IL-4 acts upon B lymphocytes, monocytes, dendritic cells, and fibroblasts and its expression (8).

Interleukin-10 is an effective anti-inflammatory and immunosuppressive cytokine that plays a vital role in preventing inflammatory and auto-immune pathologies. It has a broad range of direct and indirect effects on innate and adaptive immunity (9).

Toll-like receptor 2 (TLR2) is a member of the Toll-like receptor family and plays a significant role in the induction of innate immune cells through an MYD88-dependent pathway. It is an innate immune cell receptor that recognizes several pathogen-associated molecular patterns (PAMPs) and DAMPs and subsequently activates MYD88-dependent intracellular signaling (10). Therefore, determining the immunomodulation activity of the pure lipopolysaccharide coating of zinc oxide nanoparticles *in vivo* was the objective of this investigation.

2. Materials and Methods

2.1. Isolation and Identification of *Klebsiella pneumoniae*

One hundred and fifty samples of *K. pneumoniae* were collected from different sources in sterilized containers from different hospitals in Baghdad, Iraq (as mentioned in the previous study).

2.2. Extraction, Purification, and Binding of LPS-ZnO-Nanoparticles

LPS extraction and purification LPS was extracted by a hot phenol-water method described by Galanos, Lüderitz (11). Partial Purification of LPS by Gel Filtration (Sephacryl S-300) (12). ZnO-NPs powder was purchased from Iran, and the binding of ZnO-nanoparticles with lipopolysaccharide was done according to Li, Shi (13).

2.3. Study Design

Thirty- five female albino mice (Balb/c), aged between 8-12 weeks and 26–31.5 g weight, were used in this study. The mice were obtained from Al-Nahrain Biotechnology Research Center and kept in plastic cages, housed under a standard condition in the animal

house of the Department of Biology/College of Science/ Mustansiriyah University. The mice were left for 2 weeks for adaptation before the experiments began.

The current study included seven (7) groups, all Balb/c mice injected intramuscularly (i.m.) for 12 days as follows:

Group 1: Negative control: injected with 50 µl of normal saline. Group 2: Positive control injected with 50 µl of *Klebsiella pneumoniae* suspension equal to $<1\times10^9$ CFU/ml adjusted to 0.5 Macfarland turbidity tube. Group 3: injected with 50 µl of lipopolysaccharide (0.001µg/mouse) Group 4: injected with 50 µl of ZnO-nanoparticles (15µg/mouse) Group 5: injected with 50 µl of lipopolysaccharide ZnO-nanoparticles (1µg/mouse) Group 6: injected with 50 µl of lipopolysaccharide ZnO-nanoparticles (2µg/mouse) Group 7: injected with 50 µl of lipopolysaccharide ZnO-nanoparticles (4µg/ mouse). Also, all groups were challenged on days 4 and 8 of the injection schedule by 10% sheep RBCs (0.2ml/ mouse, i.p.). The immune response was assayed on days 11 and 12 of treatment using *in vivo* and in vitro assays.

2.4. Blood Sample Collection

After 12 days of treatment, the whole blood samples were collected in plan tubes to obtain serum, and the serum was stored at -20°C until used for immunological assays. The body weight of all the animals was measured before and after treatment in immunized albino mice treated with LPS, ZnoNPs**,** LPs-ZnoNPs, and positive and negative control groups.

2.5. Arthus and DTH Reactions

All mice in the study groups were injected with 50 µl of 10% washed SRBC in the right footpad and the left footpad injected with 50µl of normal saline as control. The thickness was measured after 4 hrs. to determine Arthus reactions and after 24 hours to determine DTH Reactions (14).

2.6. Hemagglutination Test

Hemagglutination test was performed as follows: to do this serial double-fold dilution of serum samples was made that obtained on day 12 in the 96 healthy round bottom microtiter plate by mixed 50 µl of serum in the first well with 50 µl of phosphate buffer saline and transport 50 µl to another wall which contains 50 µl PBS, then added 50 µl of 5% washed sheep red blood cells added to each well, and the plate incubated at 37 ºC for 2hrs. After the incubation period, read agglutination. Control for serum and SRBCs were made. Hemagglutination is expressed as titer: the inverse of the last dilution is positive.

2.7. Measurement of Serum Cytokines and TLRs-2 Levels

The levels of IL-1Beta, IL-4, IL-10, and TLRs-2 in the serum of mice were measured through ELISA. All the procedures were performed according to the manufacturer's instructions of Elab-science.

2.8. Statistical Analysis

The Statistical Analysis values of the investigated parameters were given in terms of Mean ± Standard Error, and the differences between means were assayed by ANOVA, and LSD test, by using the computer programmer SPSS version 20. The differences were significant when the probability value was equal to or less than 0.05.

3. Results

The present work shows the mean body weight of treated albino mice in seven 7groups before and after treatment with purified LPS, ZnoNPs and LPs-ZnoNPs. The highest value recorded in the LPs-ZnoNPs group (2 µg/mouse) is (27. 92±1.48) and (31.50±0.4) respectively, Then the highest value was in groups treated with ZnoNPs (15µg/mouse) was (27.51±1.14) and (29.9±0.72) respectively. In comparison, the LPS group (0.001 µg/mouse) was (26.5±0.82) and (28.83±0.53), the lowest value recorded in the Positive Control group (25.47±1.19) and (25.68±0.16) respectively, with a significant difference at $P\leq0.05$ (Table 1).

Table 1. The effect of LPS, ZnoNPs, and LPs-ZnoNPs on body weight in albino mice

Study groups	Body Weight Before (M±SE)	Body Weight After (M±SE)	*P*-value
Negative control 50 µl of normal saline	26.79±0.974$^{A, a}$	28.936±0.714$^{B, a}$	0.335
Positive control 50 µl of *Klebsiella pneumoniae* suspension	25.47±1.194$^{A, a}$	25.683±0.165$^{C, a}$	0.428
LPS(0.001µg/mouse)	26.5±0.824$^{A, a}$	28.833±0.538$^{B, a}$	0.245
ZnoNPs (15µg/mouse)	27.51±1.144$^{A, a}$	29.9±0.724$^{AB, a}$	0.416
LPs-ZnoNPs (1 µg/mouse)µg	27.72±1.247$^{A, a}$	30.596±0.55$^{A, a}$	0.450
LPs-ZnoNPs (2 µg/mouse)µg	27.92±1.48$^{A, a}$	31.507±0.449$^{A, a}$	0.505
LPs-ZnoNPs (4 µg/mouse)µg	27.33±1.171$^{A, a}$	30.393±0.548$^{AB, a}$	0.413
P-value	0.776	3.380379	
LSD1.11.	Non. Sign.	1.607	

M: Mean, SE: standard error, µg: microgram, ml: milliliter, P: probability. Different letters represent a significant difference between means in columns ($P \leq 0.05$), while similar letters denote non-significant differences between means in columns ($P > 0.05$)

As appeared in table 2, Arthus reaction and Delayed type hypersensitivity have revealed these results (Figure 1). The highest value was recorded in the positive control group injected with *K. pneumoniae* (4.08±0.17) and (4.86±80.02), respectively. In groups treated with LPS-ZnoNPs (4µg/mouse)**,** the highest value of Arthus reaction was (3.56±0.12) Delayed type hypersensitivity highest value was (4.23±0.09) and the lowest value recorded in the LPS group (3.30±0.07) and (3.44±0.09) respectively, other groups recorded different values of significant difference at $P \leq 0.05$.

The mean of Abs titer showed the highest value in the positive control group (551.62±7.68), followed by Group LPS (411±11.04). The lowest value was recorded in group LPs-ZnoNP in concentration 2µg /mouse (236.52±6.22) with a significant difference ($P \leq 0.05$), while the other groups recorded different values as shown in table 3.

Hemagglutination antibody titer essay is one of the parameters used to evaluate the animal's humoral immune response. In the present work, it has been found that sensitization of albino mice with 10% SRBCs stimulate the immune system of mice for the formation of antibody and high antibody titer found in LPS Group (411±11.046) that treated with 0.001µg /mouse of purified LPS, ZnoNPs was (397.72±5.679) that treated with 15 µg/mouse, and LPS-ZnoNPs was (276.2±6.475) that treated with 1 µg/mouse where at this concentration they have immune-stimulant activity on the immune system of the albino mice. On the other hand, this study found that the results of the TLR-2 showed the highest value in the positive control group (242.17±3.98) pg/ml, followed by Group LPS (135.51.58) pg/ml, and the lowest value was noted in group ZnoNPs (64.25±1.85) pg/ml with significant difference ($P \leq 0.05$) while the other groups recorded different values as shown in table 4.

Table 2. LPS, ZnoNPs, and LPS-ZnoNPs on Arthus and delayed-type hypersensitivity in immunized albino mice

Study groups	Arthus reaction (4 h)	Delayed type hypersensitivity (24 h)
	Mean ± SE (mm)	Mean ± SE (mm)
Negative control 50 µl of normal saline	2.4038±0.039^{D}	3.1332±0.026^{E}
Positive control 50 µl of *Klebsiella pneumoniae* suspension	4.081±0.175^{A}	4.8668±0.026^{A}
LPS (0.00 1µg/mouse)	3.305±0.079^{BC}	3.4476±0.095^{D}
ZnoNPs (15 µg/mouse)	3.326±0.091^{BC}	3.784±0.131^{C}
LPs-ZnoNPs (1 µg/mouse)	3.1586±0.025^{C}	3.5076±0.063^{D}
LPs-ZnoNPs (2 µg/mouse)	3.342±0.05^{BC}	3.9468±0.079^{C}
LPs-ZnoNPs (4 µg/mouse)	3.563±0.127^{B}	4.236±0.094^{B}
P-value	0.00021	0.00006
LSD	0.282059	0.237709

M: Mean, SE: standard error, µg: microgram, ml: milliliter, P: probability. Different letters represent a significant difference between means in columns ($P \leq 0.05$), while similar letters denote non-significant differences between means in columns ($P > 0.05$)

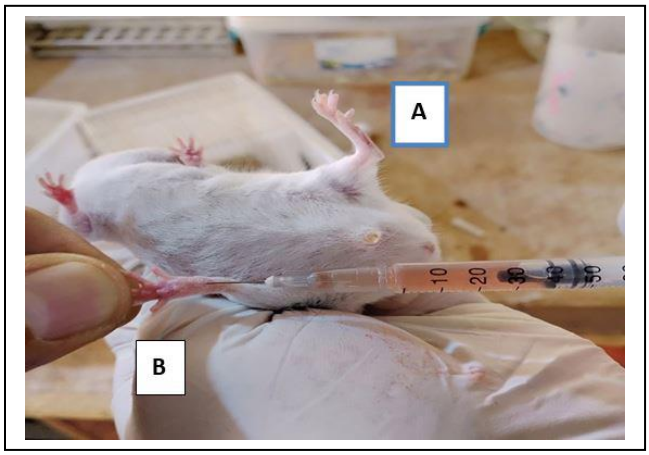

Figure 1. Delayed type hypersensitivity after 24 hours. **A:** proper footpad control. **B:** left footpad test injection SC by 50 µl of 10% RBCs

Table 3. Antibodies titer in studied groups

Study groups	Antibodies titer *
	Mean ± SE
Negative control 50 µl of normal saline	56.837±0.466^{F}
Positive control 50 µl of *Klebsiella pneumoniae* suspension	551.62±7.687^{A}
LPS (0.001µg/mouse)	411±11.046^{B}
ZnoNPs (15 µg/mouse)	397.72±5.679^{B}
LPs-ZnoNPs (1 µg/mouse)	276.2±6.475^{D}
LPs-ZnoNPs (2 µg/mouse)	236.52±6.229^{E}
LPs-ZnoNPs (4 µg/mouse)	298.13±3.135^{C}
P-value	0.00034
LSD	19.0805

M: Mean, SE: standard error, µg: microgram, ml: milliliter, P: probability. Different letters represent a significant difference between means in columns ($P \leq 0.05$), while similar letters denote non-significant differences between means in columns ($P > 0.05$).

Table 4. Serum level of TLR-2, IL-1 Beta, IL-4, IL-10 (pg/ml) in LPS, ZnoNPs and LPs-ZnoNPs treated BALB-c mice and control groups

Study groups	TLR-2	IL-1 Beta	IL-4	IL-10
	Mean±SE pg/ml	Mean±SE pg/ml	Mean±SE pg/ml	Mean±SE pg/ml
Negative control 50 µl of normal saline	17.167±0.912^{F}	71.68±1.274^{F}	26.833±0.36^{F}	63.211±0.798^{D}
Positive control 50 µl of *klebsiella pneumoniae* suspension	242.17±3.98^{A}	254.88±3.515^{A}	136.2±1.127^{A}	98.58±4.092^{A}
LPS(0.001)µg	135.5±1.583^{B}	174.3±1.462^{B}	86.12±1.498^{B}	85.14±1.082^{B}
ZnoNPs(15 µg/mouse)	64.25±1.855^{E}	101.52±1.516^{E}	44.571±0.632^{E}	71.807±0.786^{C}
LPs-ZnoNPs (µg/mouse 1)	87.167±0.912^{D}	133.9±0.584^{D}	63.202±0.347^{D}	74.66±2.271^{C}
LPs-ZnoNPs(2 µg/mouse)	85.5±1.583^{D}	130.8±0.253^{D}	61.06±0.275^{D}	82.68±1.69^{B}
LPs-ZnoNPs(4 µg/mouse)	98.83±1.824^{C}	162.4±0.579^{C}	80.405±0.249^{C}	86.018±0.692^{B}
P-value	0.00007	0.00013	0.00005	0.00012
LSD	5.921519	4.794245	2.273188	5.757758

M: Mean, SE: standard error, µg: microgram, ml: milliliter, P: probability. Different letters represent a significant difference between means in columns ($P \leq 0.05$), while similar letters denote non-significant differences between means in columns ($P > 0.05$).

The level of Interleukin-4 was determined in the serum of treated albino mice showed the highest value in the positive control group (136.2±1.12) pg/ml, followed by Group LPS (86.12±1.49) pg/ml and the lowest value was recorded in group ZnoNPs (44.57±0.63) pg/ml with significant difference ($P \leq 0.05$) while the other groups recorded different values as shown in table 4.

Interleukin 4 is associated with activation of B lymphocytes and differentiation to IgE-producing cells. IgE has a role in evoking allergy. The decline in IL-4 is a good marker that ZnoNPs (0.004 µg/mouse) and LPs-ZnoNPs (2µg/mouse) could be used in treating disease.

The results of Interleukin-10 showed the highest value in the positive control group (98.58±4.09) pg/ml, followed by LPS-ZnoNP in concentration (4µg/ mouse) (86.018±0.69) pg/ml and the lowest value was recorded in ZnoNP (71.807±0.78) pg/ml with significant difference ($P \leq 0.05$) while the other groups recorded different values as shown in table 4.

From the results of the current study, it has been found that highly significant differences were observed in IL-10 concentration in the serum of mice treated with 4 µg/mouse of the purified LPS-ZnoNPs. A significant increase in the level of IL-10 provides evidence that this cytokine plays an essential role in immune and inflammatory response towards the purified LPS-ZnoNPs, where the Expression of cytokine IL-10 contributes to reducing the inflammation. Interleukin-10 is considered a prototypic anti-inflammatory cytokine and inhibits the production of pro-inflammatory cytokines in vitro and *in vivo*.

4. Discussion

The obtained results showed that purified LPS, ZnoNPs, and LPS-ZnoNPs treated mice sensitized with SRBCs had high levels of antibodies when these mice were injected subcutaneously with SRBCs (Arthus reaction). The term refers to the inflammation that results from the deposition of immune complexes at a localized site (15). It represents an experimental model investigating the mechanism responsible for immune complex-mediated pathologies such as serum sickness or systemic lupus erythematosus. ZayedP (16) concluded that the administration of ZnO NPs in rats induces significant changes in body and organ weight and feed intake, perhaps does of ZnO NPs cause many toxicities and certain disturbances in the body.

The immunoglobulins involved in the Arthus reaction are complement-fixing IgG or IgM, and antibodies do not mediate in the DTH reaction; the DTH is dependent upon the interaction of exogenous antigens T cells, and also antigen-presenting cells all produce cytokines that stimulate a local inflammatory response in a sensitized individual. Delayed type hypersensitivity has been used as a parameter to measure the animal's cell-mediated immune response (17).

Matsui, Rouleau (18) concluded that the delayed inflammatory response in sensitive animals might be the reason for their susceptibility. Chen, Xie (19) used LPS to activate immunity in advance, significantly improving the survival rate. This coincides with the latest progress in domestication immunotherapy for leptospirosis (20). The protein levels of pro-inflammatory cytokines TNF-α and IL-1β were up-regulated in mice induced by LPS. It is reported that IL-1β and TNF-α promote the activation of macrophages and subsequent secretion of immuno-regulators, including pro-inflammatory factors that amplify the inflammatory response (21)

IL-1beta is a 17-kDa cytokine primarily produced by monocytes and macrophages and plays a crucial role in several inflammatory processes. IL-1 induces fever, expression of leukocyte adhesion molecules, hypotension, and synthesis of cyclooxygenase products. Therefore, IL-1 is a likely mediator of the systemic effects of Gram-negative infections. Although IL-l alpha and IL-l beta are distinct gene products, these two forms of IL-1 bind to the same receptors and share biological activities. A naturally occurring "IL-1 inhibitor" had been shown to block IL-1 activity by competing with IL-1 for occupancy of its receptor (22).

The inflammatory activity of LPS is mainly due to the activation of the TLR4 signaling pathway in innate

immune cells. Upon TLR4 activation, cells produce many inflammatory and stimulating cytokines such as IL1b, a crucial inflammatory immune feedback mechanism, and anti-inflammatory factors such as the IL-1 inhibitor IL-1Ra (23).

The binding of LPS to TLR4 leads to the activation of NF-_B through the recruitment and activation of MyD88, IL-1R kinase (IRAK), TNFR associated factor 6 (TRAF-6), as well as NADPH oxidase (Nox). Olejnik, Kersting (24) used different concentrations of solid ZnO. Their results showed that ZnO Nanospheres and ZnO Microrods caused low expression of some cytokines levels: IL-1a, IL-1b, IL-1ra, and IL-3. In contrast, high expression was noticed in IL-6. Other concentrations showed a variable effect on the studied parameters.

(IL-4) is an anti-inflammatory cytokine that is a 20-kDa polypeptide secreted by T cells and mast cells and has pleiotropic effects on hematopoietic cells. Human IL-4 promotes the growth of activated B and T cells, induces IgE production, and enhances the expression of B cell surface antigens, including the low-affinity receptor for IgE (FceRII/CD23) and class II major histocompatibility complex molecules (25).

The increased level of IL-10 provides evidence that this cytokine plays an essential role in immune and inflammatory responses. In contrast, the Expression of Interleukin 10 is a potent anti-inflammatory cytokine that plays an essential role in preventing inflammatory and auto-immune pathologies·IL-10, produced by the various innate and adaptive immune cells, including monocytes, macrophages, dendritic cells, B cells, and CD4+ and CD8+ T cells, IL-10 plays an important protective role. It is required to prevent bacterial dissemination and host morbidity by controlling effector T cells and the associated downstream hyperactivation of inflammatory (26).

Hanley, Thurber (27) illustrated and concluded that ZnO nanoparticles of controlled size were synthesized, and their cytotoxicity toward different human immune cells was evaluated. A differential cytotoxic response between human immune cell subsets was observed, with lymphocytes being the most resistant and monocytes being the most susceptible to ZnO nanoparticle-induced toxicity. An inverse relationship was observed between nanoparticle size and cytotoxicity, as well as nanoparticle size and reactive oxygen species production. In addition, ZnO nanoparticles induce the production of the pro-inflammatory cytokines, IFN-γ, TNF-α, and IL-12, at concentrations below those causing appreciable cell death. These results underscore the need for careful evaluation of ZnO nanoparticle effects across a spectrum of relevant cell types when considering their use for potential new nanotechnology-based biological applications. This may explain the influence of ZnO on the immune parameters used in this study (27).

Li, Shi (13) used *E. coli* LPS coated with gold (Au) NPs and examined the effects of LPS adsorption on the NP surface on the formation of a corona in biological fluids and on the subsequent inflammation-inducing activity of LPS onto the NP surface can affect the corona formation and the inflammatory properties of NPs. Thus, for an accurate interpretation of NP interactions with cells, it is essential to distinguish the intrinsic NP biological effects from those caused by biologically active contaminants such as endotoxin.

Jang, Xu (28) studied the lipopolysaccharide (LPS) coated copper sulfide nanoparticles (LPS-CuS) for immuno-photothermal therapy. They evaluated the effect of LPS-CuS for induction of apoptosis of CT26 cells and activation of dendritic cells. Moreover, the LPS-CuS and laser irradiation were examined for anti-metastasis effect by liver metastasis model mouse *in vivo*. Their results demonstrated the potential usage of LPS-CuS for immuno-photothermal therapy against various types of cancer by showing the apparent elimination of primary colon carcinoma with complete prevention of spleen and liver metastasis.

In the present study, we can conclude that LPS-ZnO NPs had a positive immunomodulatory effect on immune response in immunized mice. As shown in the

results of the level of IL-1 beta, IL-4, IL-10 and TLRs-2, Abs titer, and Arthus and DTH reactions, it may be possible to apply this complex as new nanoparticle-based vaccine adjuvants or immune therapeutics.

Authors' Contribution

Study concept and design: F. S. A.
Acquisition of data: I. K. A.
Analysis and interpretation of data: N. H. Z.
Drafting of the manuscript: F. S. A
Critical revision of the manuscript for important intellectual content: I. K. A.
Statistical analysis: N. H. Z.
Administrative, technical, and material support: F. S. A.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgment

We want to thank the Iraqi Ministry of Higher Education and Scientific Research, Mustansiriyah University, as well as Iraqi Ministry of Health educational laboratories in medicine city in Baghdad, and Al- Iraq specialized laboratory for participating in the study.

References

1. Sharma S, Mudgal N, Sharma P, Shrngi B. Comparison of phenotypic characteristics and virulence traits of Klebsiella pneumoniae obtained from pneumonic and healthy camels (Camelus dromedarius). Adv Anim Vet Sci. 2015;3(2):116-22.
2. Yu W-L, Chuang YC, Calderwood SB. Microbiology and pathogenesis of Klebsiella pneumoniae infection. UpToDate: UpToDate, Waltham, MA. 2008.
3. Tan Y, Kagan JC. A cross-disciplinary perspective on the innate immune responses to bacterial lipopolysaccharide. Mol Cell. 2014;54(2):212-23.
4. Ju-Nam Y, Lead JR. Manufactured nanoparticles: an overview of their chemistry, interactions and potential environmental implications. Sci Total Environ. 2008;400(1-3):396-414.
5. Madhumitha G, Elango G, Roopan SM. Biotechnological aspects of ZnO nanoparticles: overview on synthesis and its applications. Appl Microbiol Biotechnol. 2016;100(2):571-81.
6. Turner MD, Nedjai B, Hurst T, Pennington DJ. Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease. Biochimica et Biophysica Acta (BBA)-Molecular Cell Res. 2014;1843(11):2563-82.
7. Gibson MS, Kaiser P, Fife M. The chicken IL-1 family: evolution in the context of the studied vertebrate lineage. Immunogenetics. 2014;66(7):427-38.
8. Reinhart R, Kaufmann T. IL-4 enhances survival of in vitro-differentiated mouse basophils through transcription-independent signaling downstream of PI3K. Cell Death Dis. 2018;9(7):1-12.
9. Mingomataj EC, Bakiri AH. Regulator versus effector paradigm: interleukin-10 as indicator of the switching response. Clin Rev Allergy Immunol. 2016;50(1):97-113.
10. Bagheri V, Askari A, Arababadi MK, Kennedy D. Can Toll-Like Receptor (TLR) 2 be considered as a new target for immunotherapy against hepatitis B infection? Hum Immunol. 2014;75(6):549-54.
11. Galanos C, Lüderitz O, Westphal O. A new method for the extraction of R lipopolysaccharides. Eur J Biochem. 1969;9(2):245-9.
12. SH R, Mohammad S, Sameer E. A partially purified lipopolysaccharide extracted from klebsiella pneumonia as antibacterial. Extraction.52:36.1.
13. Li Y, Shi Z, Radauer-Preiml I, Andosch A, Casals E, Luetz-Meindl U, et al. Bacterial endotoxin (lipopolysaccharide) binds to the surface of gold nanoparticles, interferes with biocorona formation and induces human monocyte inflammatory activation. Nanotoxicology. 2017;11(9-10):1157-75.
14. Tansho S, Abe S, Mizutani S, Ono Y, Takesako K, Yamaguchi H. Protection of mice from lethal endogenous Candida albicans infection by immunization with Candida membrane antigen. Microbiol Immunol. 2002;46(5):307-11.
15. Levinson W. Review of medical microbiology and immunology: McGraw-Hill Education; 2014.
16. ZayedP NA. Effect of ZnO NPs on body and organ weights in male rats. IJISET. 2018;5(8):12-25.
17. Meier R, Bein H, Jaques R. The action of bacterial polysaccharides on allergic phenomena. Int Arch Allergy Immunol. 1957;11(1-2):101-18.

18. Matsui M, Rouleau V, Bruyère-Ostells L, Goarant C. Gene expression profiles of immune mediators and histopathological findings in animal models of leptospirosis: comparison between susceptible hamsters and resistant mice. Infect Immun. 2011;79(11):4480-92.
19. Chen X, Xie X, Wu D, Zhang S, Zhang W, Cao Y. The pre-activated immune response induced by LPS protects host from leptospirosis. Plos One. 2020;15(11):e0242742.
20. Santecchia I, Vernel-Pauillac F, Rasid O, Quintin J, Gomes-Solecki M, Boneca IG, et al. Innate immune memory through TLR2 and NOD2 contributes to the control of Leptospira interrogans infection. PLoS Pathog. 2019;15(5):e1007811.
21. Cagliero J, Villanueva SY, Matsui M. Leptospirosis pathophysiology: into the storm of cytokines. Front Cell Infect Microbiol. 2018;8:204.
22. Dinarello CA. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. Blood. 2011;117(14):3720-32.
23. Murphy K, Weaver C. Janeway's immunobiology: Garland science; 2016.
24. Olejnik M, Kersting M, Rosenkranz N, Loza K, Breisch M, Rostek A, et al. Cell-biological effects of zinc oxide spheres and rods from the nano-to the microscale at sub-toxic levels. Cell Biol Toxicol. 2021;37(4):573-93.
25. Shirley M. Dupilumab: first global approval. Drugs. 2017;77(10):1115-21.
26. Kamanaka M, Kim ST, Wan YY, Sutterwala FS, Lara-Tejero M, Galán JE, et al. Expression of interleukin-10 in intestinal lymphocytes detected by an interleukin-10 reporter knockin tiger mouse. Immunity. 2006;25(6):941-52.
27. Hanley C, Thurber A, Hanna C, Punnoose A, Zhang J, Wingett DG. The influences of cell type and ZnO nanoparticle size on immune cell cytotoxicity and cytokine induction. Nanoscale Res Lett. 2009;4(12):1409-20.
28. Jang B, Xu L, Moorthy MS, Zhang W, Zeng L, Kang M, et al. Lipopolysaccharide-coated CuS nanoparticles promoted anti-cancer and anti-metastatic effect by immuno-photothermal therapy. Oncotarget. 2017;8(62):105584.