

The Promoting Role of Zinc Oxide Nano Particles (ZnO-NPs) Enhancing the Immunogenic Activity of *Escherichia coli* Lipopolysaccharides (ELPS) in vivo

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Abstract

Escherichia coli (*E. coli*) is considered one of the microorganisms that characterized as a double edge sword by their pathogenic role as well as commensal role. Lipopolysaccharides (LPS) is considered one of the endotoxins which are secreted by gram negative bacteria including *E. coli* Nanoparticles (NPs) are natural metals that have the ability to access through such a different micro spaces area in the body suggesting the drug delivery function of these NPs. In this current study, after uploading the LPS on the ZnO, The results of the current study suggesting the delivery role of zinc oxide giving the rise for LPS to access through more larger area surfaces in the body which leading to significant immune response of different parameters in the body including body temperature , pyrogenic activity , Antibody titration as well as the level of the proinflammatory cytokines (TNF α) compared to LPS alone. Taken together, our data indicated a significant role for the Nanoparticles particularly zinc oxide promoting the antigenic and pathogenic role of LPS compared to LPS alone, this may suggest the future role that these nanoparticles may be playing through delivering other natural compounds or drug in a consistent doses which may increasing the role of NPs in the field of drug delivery and drug safety which leading to improve the outcome of the treatment.

Keywords:

ELPS, Immune response, Nanoparticles, Zinc Oxide

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Introduction

E. coli is considered as a remarkable microorganism which considered in charge of causing wide range of several diseases that range from gastroenteritis to external gastrointestinal tract infections such as a urinary tract infection, blood stream infection and central nervous system diseases (Croxen and Finlay, 2010).

Glycolipid which is known as lipopolysaccharides (LPS) is considered one of the cell surface molecules that are produced by gram negative bacteria giving rise for stimulating immune response (Bertani and Ruiz, 2018).

Nanoparticles (NPs) which are biologically and chemically characterized by different forms that range from dispersions particulate to solid particles, as well as a characterization in different sizes that range from 10 -1000 nm (De Jong and Borm, 2008).The efficiency and the significance of using nanoparticles is due to their properties and unique role enhancing their parent material (Altammar, 2023).

Zinc oxide nanoparticles (ZnO-NPs) are an oxide of metals that may provide an opportunity for medical applications especially in the diagnosis and treatment of different diseases. Zinc specifically is found in the muscle, bone, skin and teeth which is in general recognized as a safe substance (Baek et al., 2012).

In order to make a relationship between the nanoparticles and lipopolysaccharides, so this study have been designed to investigate the role and efficacy of the nanoparticles through their accessibility, uploading and delivery role of the antigens experimentally including lipopolysaccharides to more surface of the different organs in the body host which

leading to improve the antigenicity and immune reactivity toward lipopolysaccharides leading in this case to know more scientific facts about the bio-immunological role of the lipopolysaccharides.

Material and methods

Ethical approval

All methods were followed in ethical dealing with animals, according to the instructions of the Ministry of Higher Education and Scientific Research in Iraq.

Lipopolysaccharide (LPS) extraction

The extraction was done by using saturated phenol with some modifications (Davis and Goldberg, 2012).

Loading of LPS

LPS was loaded on zinc oxide nanoparticles with a concentration of 300µg/ml while the concentration of LPS which was used in this study was 10 mg/ml.

Animals:

Adults rats were used in this study with age of 10 weeks which obtained from the Animals House in the College of Veterinary Medicine at Tikrit University.

Experimental animal groups:

The study were designed into three groups, each group consists of 4 rats which include control or naïve group that were injected with normal saline with a volume of 200µl and the second group which were injected with LPS alone with a dose of 5mg/kg and third group that was injected with LPS 10mg/kg which was uploaded on zinc oxide nanoparticles with a concentration 300µg/ml.

Body temperature was then measured after 4 hrs using a thermometer through the rectum of the rats.

Detection of Humeral immunity:

In order to measure the humeral immunity, agglutination test was done by using an antigen prepared according to (Kwapiniski, 1972).

Detection of Cellular immunity:

A- In order to detect the cellular immunity, phagocytic index was used based on (Metcalf et al., 1986).

B- Enzyme Linkage Immune Sorbent Assay (ELISA)

TNF α was measured using TNF α Kits, Cusa-bio company.

Statistical Analysis:

The statistical software which was used in this study Graph Pad Prism 6.0 Software (San Diego, CA, United States) Student's t-test was used for comparison among two groups. One way-ANOVA or multiple comparisons of T test with post hoc Tukey's test was used to compare between more than two groups. The results were expressed as mean \pm SEM. p -value \leq

of 0.05 was considered statistically significant.

Results

The results of this study were showing that the body temperature was increasing in the LPS group compared with Naïve group while the temperature significantly decrease in the LPSNPs group compared to LPS alone as listed (Fig. 1).

Another parameter which is the titration of the antibody where the results of this study has been shown that the this parameter has been increased in the LPS group compared to naïve group , while the results in the LPS NPs were increased more compared with LPS alone (Fig. 2).

On the other hand, another parameter which is the phagocytic index, and it has been shown that this index has been significantly increased in the LPS group, where these results are increasing more significantly in the LPSNPS compared to LPS alone (Fig. 3).

The results of the ELISA kit have been shown a significant increase in the LPS group compared to the Naïve group while the level of TNF α in serum has been increased more significantly in LPSNPS group compared to LPS alone (Fig. 4).

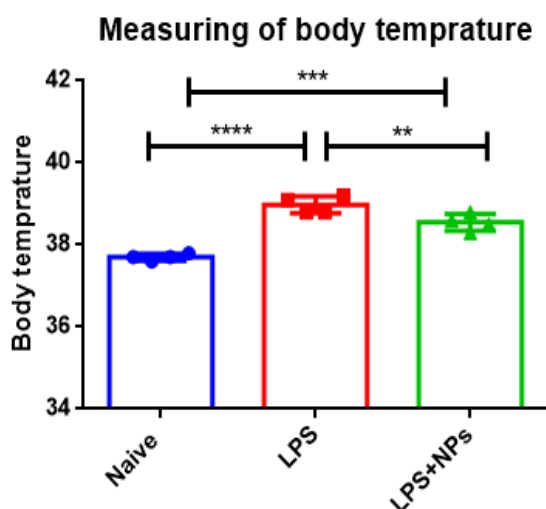


Fig. 1. Measurement the difference in the body temperature. Five rats were included in each group between 3 groups including Naïve group, LPS group and LPSNPs group were used. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

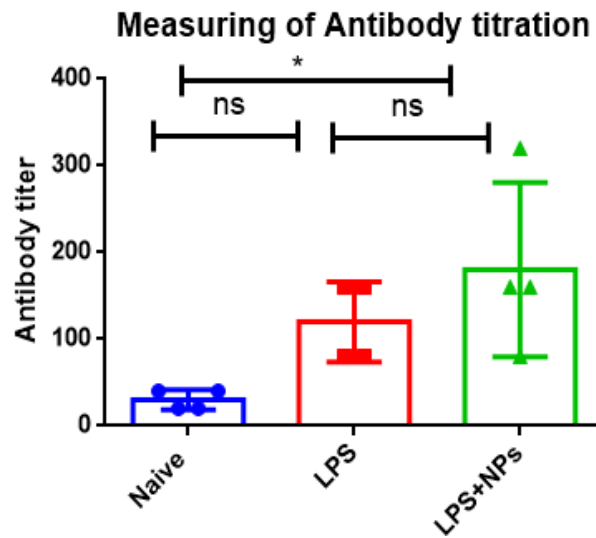


Fig. 2. Measurement the difference for the titration of antibody, five rats were included in each group between 3 groups including Naïve group, LPS group and LPSNPs group were used. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

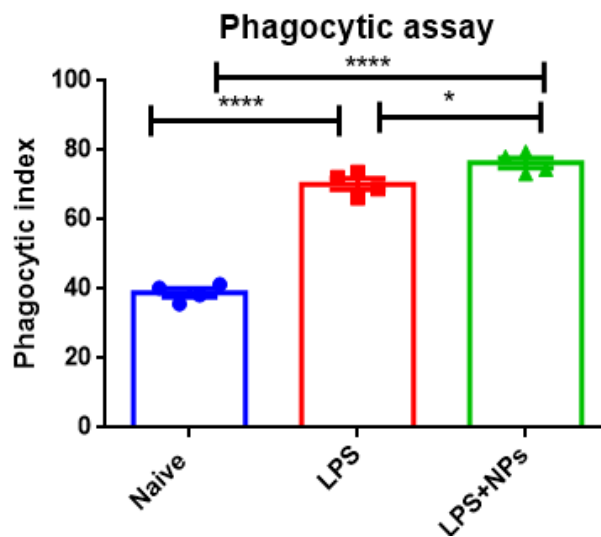


Fig. 3. Measurement the phagocytic assay, five rats were included in each group between 3 groups including Naïve group, LPS group and LPSNPs group were used. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

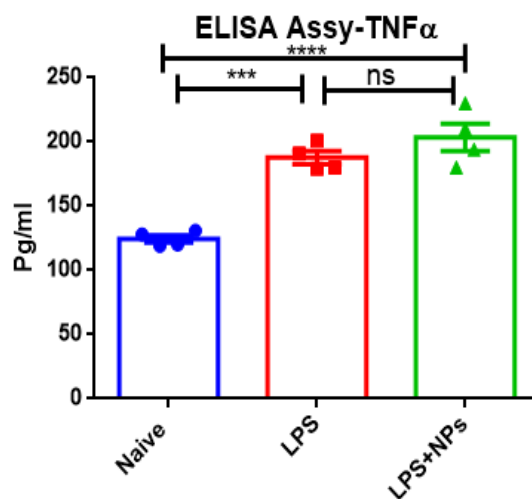


Fig. 4. Measurement the difference for the level of cytokines, five rats were included in each group between 3 groups including Naïve group, LPS group and LPSNPs group were used. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

Discussion

Escherichia coli is a gram negative, causing an infection in the human being, while the lipopolysaccharides are considered one of the virulence mechanisms, giving the promotion's right for the infection (Luthje and Brauner, 2014).

Our investigation in this study has been focusing on an important spot for the nanoparticles (NPs), particularly Zinc Oxide (ZnO), a delivery agent facilitating the delivery of ELPS leading to enhancing of immune response, which suggesting the role of these NPs to access through larger surface area inside the body and giving rise of more clear picture related understanding the immunopathogenic role of LPS. The results of the current study suggested that LPS has been significantly influenced on the body temperature in the LPS group compared to naïve group, which may suggest the efficacy of LPS inducing immune response and pathogenicity (Habicht, 1981).

Based on the data listed in Fig. 1, which suggested a significant increase in the LPS group compared with naïve group, the

results of our study show that there is a significant decrease in the body temperature in the LPSNPs compared with LPS alone treated with Zn-ONPs, which may explain that nanoparticles may deliver the LPS leading to induce the immune response but not necessarily increasing the body temperature.

Humoral immunity is considered one of the immune responses which induced by LPS exposure (Baldi et al., 1996). The results of the current study indicated an increase in the titration of the antibody in the LPS group compared with Naïve group, while the titration was higher in the LPSNPs group compared with LPS alone. This finding may suggest the effective role of both LPS and NPs respectively, which is identified with other study that indicated Nanoparticles enhancing the humoral immunity through promoting central B cells, long lived plasma cells and memory B cells, which associated with protective antibodies in mice (Alameh et al., 2022).

TNF α is a proinflammatory cytokine is responsible of wide range of signaling events in the cells (Idriss and Naismith, 2000; Papadopoulos, 2024). The results obtained from this study has been showing

a significant increase in the LPSNPs group compared to LPS alone, suggesting the accessory role of NPs delivering the LPS to more micro spaces area in the body of rats. These results are in consistent with another study that suggested the accurate role of NPs in promoting the outcome of the LPS (Shetab Boushehri et al., 2018).

Conclusion

In conclusion, the results of the current study indicated a significant increase in the level of immune response in the LPS group compared with naïve group. While, the LPS group treated with nanoparticles were there more immune response, suggested the efficacy of nanoparticles. These results showed a significant increase in the LPS group compared with naïve group, demonstrating of the efficacy of LPS. While, the immune response became more severe in the LPS group loaded on nanoparticles, suggesting the efficacy of NPs delivery function into super micro spaces accessible area that cannot be reached by LPS alone .This suggesting the role of Zinc oxide and their function as a drug delivery and potentially use in vaccine applications against *E. coli* infection in the future.

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