



The effect of zinc oxide nanoparticles on blood parameters and immunoglobulin A in male rats with diarrhea induced

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Abstract

Objective: The present study was designed to detect the ability of ZnO NPs to inhibit the bacterial isolates that cause diarrhea in laboratory male rats and determination the blood parameters, liver enzyme functions and IgA levels after treatment with ZnO NPs. **Material and methods:** Twenty rats of 6 weeks-old male were used and divided as follow: Group 1: Control group that were not treatments. Group 2: The animals were infected with 1.2×10^{-8} of *Clostridium difficile*, (Infected group). Group 3: The rats were infected with *C. difficile* and orally dosage with 150mg/kg from ZnO NPs for two weeks. **Results:** The results showed that the orally dosage from *C. difficile* was causing the significant ($P < 0.05$) decreased in RBCs, Hemoglobin concentration (Hb), HCT, and significant increase of WBCs count, neutrophil and decreased the lymphocytes count cells. Also, it was caused in significantly increased of ALT, AST and ALP enzymes in serum and IgA levels compare with same values in control group. the orally dosage from ZnO NPs was done all parameters were come at similar of the same parameters in control group. **Conclusion:** It's concluded that ZnO NPs has ability to potential inhibition activity against *C. difficile* that induced diarrhea.

Keywords: *C. difficile*, ZnO NPs, liver enzyme, IgA

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INTRODUCTION

Nanotechnology is a research crucial point in recent materials science. This technology is able to provide various version applications that domain from innovative fabric compounds, food processing, and agricultural production to sophisticated medicinal techniques (Sahoo, 2010; Nyakora, O2016, Kumar, & SINHA, 2017). The properties and functions of living and anthropogenic systems are defined (Yadavet, al. 2013, Hussein, et al. 2017). In this technology, the relevant materials who structures show new and increase physicochemical and biological properties in addition special phenomena and functionalities as a result of the nanoscale size (Pal, et al. 2007). The nanoscale size mostly gives larger surface areas to nanoparticles (NPs) compared with macro-sized particles (Ashe, 2011). NPs are known as controlled or manipulated particles at the atomic level between 1 to 100 nm. They appear size-related properties significantly different from bulk materials (Bulusu, et al. 2000). Small size of NPs has larger structures in comparison with counterparts. These distinct properties allow their possible applications in much fields as biosensors, nanomedicine, and bio

nanotechnology (Sahoo, 2010. Edwards, et al. 2010) *Clostridium difficile* (also known as *Clostridioides difficile*) is the most common pathogen causing health care-associated infections in the United States, accounting for 15% of all such infections. A Centers for Disease Control and Prevention report on antibiotic resistance threats categorized *C. difficile* as an urgent threat. Antibiotic treatment for *C. difficile* infection (CDI) is often followed by recurrent infection, leading to nontraditional treatments, such as fecal transplant and oral administration of nontoxicogenic *C. difficile* spores

The aim of study was to determine the ability of orally dosage from ZnO NPs on the blood profile and liver enzyme activity and immunoglobulin-A in the serum of laboratory rats induced diarrhea by infection with *C. difficile*.

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Table 1. Effect of ZnO NPs on hematological parameters of male rats induced with diarrhea used *C. difficile*

TRT	Hb (g/dl)	RBCs ($\times 10^6/\text{mm}^3$)	Hct.(%)	WBCs ($\times 10^6/\text{mm}^3$)	Neut. (%)	Lym.(%)
Group A	12.7 \pm 0.14 a	5.83 \pm 0.84 a	39.4 \pm 0.6 a	12.25 \pm 2.9 b	61.2 \pm 3.4 c	35.2 \pm 1.6a
Group B	8.55 \pm 0.48 b	3.41 \pm 0.61 b	24.35 \pm 2.2 b	15.77 \pm 1.7 a	72.3 \pm 4.1 a	25.7 \pm 1.5c
Group C	13.55 \pm 0.21 a	5.27 \pm 0.94 a	41.5 \pm 0.7 a	12.65 \pm 1.6 b	64.9 \pm 3.7b	31.4 \pm 2.6b

Group-A=control, Group-B=Infected with diarrhea at 1×10^8 of *C. difficile*, Group-C=Treatment with 150mg/kg ZnO NPs.

MATERIAL AND METHODS

Animals Model

Twenty four rats of 6 weeks-old male (Albino-Sprague Dawley Rats) obtained from the laboratory animal house in veterinary college in Tikrit University. The rats were individually weighed; wing banded and housed in heated battery brooders under 12 hrs. fluorescent lighting daily with feed and water provided *ad libitum* and the temperature was between 23-25 ° C. Rats were fed the basil diet formula (NCR, 1992). Rats were placed under observation by a veterinarian for 5 days prior to the experiment, to ensure of rat's health.

Experiment design

Twenty four of male Rats were randomly assigned to three groups, each one contains two replicates, each replicates were consisted four rats, and the distribution were as follows:

- ❖ Group 1: the animals were not treatments, (Control negative).
- ❖ Group 2: The animals were infected with *C. difficile*, (Control Positive).
- ❖ Group 3: The animals were infected with *C. difficile* and treatment by orally dosage by 150mg/kg of ZnO NPs for two weeks.

The experimental was distributed to three stages that contains:

- Initial period: in this period the rats were placing for 5 days under observation, and then the blood was withdrawn on the eighth day and was considered as control negative.
- Infection period: rats were orally dosage from the infection bacteria of *C. difficile* at 1.2×10^8 cell/ ml, till the symptom of infection as diarrhea were appearing on most of the rats.
- Treatment period: The rats which infected with *C. difficile* were treatment by orally dosage by 150mg/kg of ZnO NPs for two weeks.

Parameters assay

At the last of the experiment, five milliliters of blood were collected from eyes artery of the treated rats, then dispensed in two tubes, one of its with EDTA for hematological parameters, while the second tubes were without EDTA for biochemical tests determination. Hematological parameters include: hemoglobin Hb, Total red blood cells RBCs and total leucocyte counts TLC. Biochemical parameters include: AST, ALT and Alkaline phosphatase AIP. Serum immunoassay for IgA antibody immunoassay was performed with Shanghai kit

and comparison of pre-treatment and post-treatment values.

Statistical Analysis

The experiment was performed under the complete Randomized Design, and the variance analysis was carried out using the General Linear Model within the minitap 17 (Statistical Analysis System). Duncan (Theml, et al. 2004). was used in the case of significant differences between the different averages at level 0.05.

RESULTS

Effect of ZnO NPs on blood profile: The results in **Table 1** were illustrated the effects of orally dosage from *C. difficile* alone or when treatment with the Zn NPs on hematological parameters in male rats animals. The results showed that the orally dosage from *C. difficile* was causing the significant ($P < 0.05$) decreased in the hemoglobin concentration and RBCs and became at 8.55 g/dl and 5.83 ($\times 10^6/\text{mm}^3$) respectively compared to the control group which non treatment with the *C. difficile* isolates (12.7g/dl and $3.41 \times 10^6/\text{mm}^3$ respectively). The orally dosage of the Zn NPs at 150mg/kg was caused in efficacy of the Hb and RBCs levels at 13.55 and 5.27 $\times 10^6/\text{mm}^3$ respectively which were moderate the level of Hb to become near of the value in the control group (12.7g/dl). The results also showed the current study that experimental bacterial infection with *C. difficile* has caused a significant reduction in the percentage of Hct (24.35), but in the treated groups show improved in percentage of Hct. Also, about total count of WBCs, neutrophil and lymphocyte percentages which show significant increased to 15.77 ($\times 10^6/\text{mm}^3$), 72.3 and 25.7% respectively compare with control group 12.25 ($\times 10^6/\text{mm}^3$) 61.2 and 35.2 % respectively, but in the treated group was show improved in the total count of WBCs, neutrophil and lymphocytes percentages and the values became near or similar to the control rats group as shown in **Table 1**.

The treatment of male rats with ZnO NPs, was caused in significantly decreased of the WBCs count and increased of RBCs and platelets counts and Hb% when compared to control groups. these results were agreement with study by (Ashe, 2011). Whom found that *C. difficile* is caused of diarrhea and pseudo membrane colitis leading to cause of nosocomial diarrhea and the leukocytosis was increased with *C. difficile* positive patients to 15,800 ($\times 10^6/\text{mm}^3$), and caused iron deficiency with hemoglobin concentration < 11 g/dl. In the current study, a decrease in the white blood cell count and an increase in Hb, HCT and R.B.C

Table 2. Effect of ZnO NPs on liver enzymes activity in male rats induced with diarrhea used *C. difficile*

Treatments	AST (U/L)	ALT (U/L)	ALP (U/L)
Group-A	41.1±2.15b	45.3±3.57b	72.7±4.51c
Group-B	61.33±6.43 a	65.33±10.21 a	91.33±3.24 a
Group-C	39.0±7.0 b	44.0±5.57 b	75.4±2.7 b

Group-A=control, Group-B=Infected with diarrhea at 1×10^6 of *C. difficile*, Group-C=Treatment with 150mg/kg ZnO NPs

levels were observed when using ZnO NPs in treating the male rats was agreement with (Ali Alkaladi, et al. 2015). Imen Ben Slama (2015). Nema A et al. 2019). whom show that ZnO NPs effected on these parameters to became similar of the value with the control group.

Effect of ZnO NPs on Liver function enzymes: The results in **Table 2** was investigate the effects of ZnO NPs on the liver enzymes of male rats that induced diarrhea infection by *C.difficile*.

The results were found that the levels of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), and Alkaline phosphatase (ALP), were significantly ($p < 0.05$) increased and became at 61.33, 65.33 and 361.33 U/L respectively with the male rats infected with diarrhea compared with these levels in control rats group which at 41.1, 45.3 and 72.7 U/L respectively. The treatment male rats with ZnO NPs at 150 mg/ml were caused in significantly decreased of liver enzyme and became at 39.0, 44.0 and 75.4 U/L respectively, and were caused improved the animal health. These results were consistent with its findings of (Cui, et a al. 2014 Fathi, 2016). Samir et al. 2017). whom reported that treatments with ZnO NPs was caused in improved the levels of enzymes in rats induced with diarrhea.

Table 3. Effect of ZnO NPs on immunoglobulin-A in male rats induced with diarrhea used *C. difficile*

Treatments	IgA
Group-A	14.6±0.61b
Group-B	34.5±4.95a
Group-C	15.1±9.19b

Group-A=control, Group-B=Infected with diarrhea at 1×10^6 of *C. difficile*, Group-C=Treatment with 150mg/kg of ZnO NPs

Effect of ZnO NPs on IgA level:

The orally dosage effects of 150 mg of ZnO NPs on the immunoglobulin-A of laboratory rats serum that induced diarrhea infection with *C. difficile* was illustrated in **Table 3**. The results were indicated that orally dosage from ZnO NPs were caused in efficacy of the IgA levels (15.1 $\mu\text{g/ml}$) to similar value and not significantly differences with the control group which was at 14.6 $\mu\text{g/ml}$ while the IgA value in the serum of rats which induced infected with diarrhea was at 34.5 $\mu\text{g/ml}$.

This result was closely agreement with (Priscilla et al. 2015. Zhanga. et al. 2017). Xun Pei,et al. 2018). whom used of ZnO NPs in treatment of diarrhea in rats, and them found the nanoparticules was effected on induce peripheral immune reaction in laboratory rats and trigger adaptive immunity through the innate immune system turn IgA levels decreased after inhibitions the pathogen which caused the infections.

CONCLUSION

It's concluded that ZnO NPs has ability to potential inhibition activity against *C. difficile* that induced diarrhea.

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