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RESEARCH ARTICLE

Therapeutic effects of bioconjugated Linalool-zinc oxide nanoparticles against *Giardia lamblia* infection through modulating serum electrolytes and inhibiting inflammation

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ABSTRACT

Nowadays, there has been a noticeable rise in the utilization of nanoparticles in a diverse array of fields, including medicine and industry. The current research seeks to evaluate the *in vivo* therapeutic efficacy of bioconjugated Linalool-zinc oxide nanoparticles (ZOP) in the treatment of *G. lamblia* infection. The impact of Linalool-ZOP at dosages of 20 mg/kg and 40 mg/kg, both individually and in conjunction with metronidazole (MTZ, 7.5 mg/kg) on the number and viability of *Giardia* cysts, the serum level of electrolytes of sodium (Na+) and potassium (K+), as well as the NF-κB signaling-related genes ((Tumor Necrosis Factor-alpha (TNF-α), Interleukin-1 (IL-1), IL-10, Nuclear Factor kappa B p65 (NF-κB p65), and Toll-like Receptor 4 (TLR4)) were assessed. We found that the average diameter of Linalool-ZOP was determined to be 105 nm. Following a seven-day treatment of *G. lamblia*-infected mice with Linalool-ZOP mainly in conjunction with MTZ, the number and viability of *G. lamblia* cysts was significantly decreased (P<0.001). Linalool-ZOP, particularly in combination with MTZ, notably modulated the serum levels of Na and K in the infected mice (P<0.001). The Linalool-ZOP, particularly in conjunction with MTZ independently led to a notable drop in the *TNF-α, IL-1, NF-κB p65, and TLR4* genes, as well as a marked increase in *IL-10* gene expression (P<0.001) with no toxicity on vital organs in mice. The present study revealed that the Linalool-ZOP, mainly in combination with MTZ, significantly alleviated *Giardia* infection in murine models by reducing inflammation and rectifying serum electrolyte imbalances. Should additional mechanisms be clarified and subsequent clinical trials involving human subjects produce positive outcomes, these compounds could be considered potential candidates for developing a new therapeutic approach for giardiasis. Furthermore, we advocate for the initiation of human clinical trials and a more comprehensive assessment of the toxicity of Linalool-ZOP in more intricate models.

Keywords: giardiasis; nanomedicine; anti-inflammatory; diarrhea.

INTRODUCTION

Giardia lamblia as a protozoan parasite found in the human small intestine and is prevalent worldwide, with contamination rates ranging from 1 to 25% across different regions (Innes, 2010). It is estimated that there are approximately 280 million human infections globally each year. Transmission to humans, particularly children, occurs through via the ingestion of infectious forms present in contaminated food and water sources (Laishram *et al*., 2012). Clinical manifestations of *G. lamblia* infection include gastrointestinal complications such as diarrhea, foul-smelling and fatty stools, stomach upset or nausea, malabsorption, and bloating (Laishram *et al*., 2012). Given the absence of a dependable and secure vaccine, currently regarded as the most effective approach for managing giardiasis (Watkins & Eckmann, 2014). Treatment typically involves the administration of one of four drugs: metronidazole, furazolidone, tinidazole, and quinacrine, all of which have associated side effects and uncertain efficacy (Watkins & Eckmann, 2014). Although these drugs are typically efficacious, they are associated with negative outcomes, including unpalatable flavor and gastrointestinal issues nerve damage, agitation, seizures, and vertigo, which may impede the therapeutic regimen as well as drug resistance and mutagenic effects (Vivancos *et al*., 2018). As a result, there is an urgent requirement to identify a pharmaceutical agent that exhibits a low incidence of adverse effects.

Nowadays, nanoparticles (NPs) are widely used in a diverse array of fields, including medicine and industry (Formoso *et al*., 2016). Nanoparticles offer a diverse array of applications, particularly in the medical field, and have shown significant progress in improving the efficacy and distribution of pharmaceuticals, while simultaneously minimizing adverse effects associated with medication, and streamlining the production of nanomaterials (Rohela *et al*., 2019). NPs are esteemed for their beneficial attributes, including heightened bioavailability, increased solubility through

hydrophobic activation, enhanced pharmacokinetics of active pharmaceutical ingredients, and diminished toxicity, rendering them valuable for medical and pharmaceutical purposes (Albalawi *et al*., 2020). Metal nanoparticles due to exceptional characteristics, are commonly utilized as an effective means for the delivery of both small drug molecules and larger biomolecules (Rohela *et al*., 2019). Zinc oxide nanoparticles (ZOP) have attracted considerable interest in the field of nanomaterials. due to their diverse potential applications in the medical field, such as their ability to serve as anticancer, anti-inflammatory, and antimicrobial agents (Moezzi *et al*., 2012). The rapid advancement of nanotechnology has opened up new avenues for utilizing natural products, leading to numerous research endeavors exploring the medical applications of nanomaterials incorporating natural substances (Watkins *et al*., 2015). Linalool ($C_{10}H_{18}O$) is a monoterpenoid compound derived from a variety of plant sources, recognized for its therapeutic properties. It has been associated with analgesic effects, anti-inflammatory activity, cancer inhibitory potential, and the suppression of microbial growth, as evidenced in therapeutic practices (Aprotosoaie *et al*., 2014). The current research seeks to evaluate the *in vivo* therapeutic efficacy of bioconjugated Linalool-ZOP for *G. lamblia* infection treatment.

MATERIALS AND METHODS

Bioconjugated Linalool-ZOP

Linalool-ZOP were synthesized by combining ZNP, $Zn(NO_3)_2{\cdot}4H_2O$, PVA (0.01%), and NaOH (0.5 M) (Sigma Aldrich, USA) using a magnetic stirrer for a duration of 240 minutes, as detailed in our previous research (Albalawi *et al*., 2024) and in accordance with the previous methodology (Mirhosseini & Firouzabadi, 2013).

Physical and chemical characteristics of Linalool-ZOP

The physical characteristics of Linalool-ZOP, including their dimensions and morphology, will be evaluated utilizing a scanning electron microscope (SEM) (Mira3, manufactured in the Czech Republic) and a Zetasizer device (Malvern, UK). The crystalline structure of the Linalool-ZOP will be determined using a UV–visible spectrophotometer (JENWAY 6405). Furthermore, the verification of the green synthesis of the nanoparticles via extract will be conducted through X-ray diffraction (XRD) analysis (2000 APD, Italy). The surface chemistry of the biogenic Linalool-ZOP will be analyzed using a Fourier-transform infrared (FTIR) spectrophotometer (Shimadzu IR-470, Japan) with a resolution of 40 mm, employing potassium bromide disks for the analysis.

Parasite

G. lamblia provided from Tabuk university (ATCC 30957) were cultured in TYI-S-33 medium (Merck, Germany) improved with 20% FCS (Merck, Germany), bovine bile (Merck, Germany), strep/pen (1%) and maintained at 37°C. The quantity of cysts was subsequently calibrated to 100000 cysts per mL utilizing a Neubauer chamber (Malekifard *et al*., 2020).

Therapeutic effects of Linalool-ZOP on giardiasis in mice

Animal

In this study, forty-eight NMRI mice aged between 6 and 8 weeks (20 to 25 g), were utilized. The mice were maintained under standardized environmental conditions, 24 ± 1°C, a 12-h light/dark cycle, and 40-70% humidity *ad libitum*.

Ethics

The present study is approved by the Ethics Committee of the Shaqra University, Saudi Arabia under the ethical approval code of ERC-SU-F-20250001.

Establishment of giardiasis in mice

To induce giardiasis in a cohort of eighty mice, an oral dose of 0.5 mL containing 2×10^4 cysts of *G. lamblia* was administered. The presence of infection was assessed through daily microscopic examinations, which began on the day of infection and continued until the detection of cysts in the feces, utilizing the formalin-ether concentration method (Albalawi & Alanazi, 2023). The induction of giardiasis was confirmed in all subjects by the third day following the oral administration of *G. lamblia* cysts.

Study design and treatment

Following the confirmation of infection in all subjects, animals were allocated to six groups in a random manner, each consisting of eight individuals. The experimental subjects received treatment with the designated agents over a continuous duration of seven days (Albalawi & Alanazi, 2023), which included the following conditions:

i. Administered Normal saline
ii. Administered with Metronidazole (MTZ) at 15 mg/kg (Albalawi & Alanazi, 2023).
iii. Administered with Linalool-ZOP at 20 mg/kg
iv. Administered with Linalool-ZOP at 40 mg/kg
v. Administered with MC (7.5 mg/kg) + Linalool-ZOP at 20 mg/kg
vi. Administered with MC (7.5 mg/kg) + Linalool-ZOP at 40 mg/kg

Parasitological assessment

After a seven-day treatment period, fecal samples from the mice were subjected to analysis via the formalin-ether method to identify the presence and number of cysts. Furthermore, the viability of the excreted cysts was reviewed via eosin staining (0.1%). Cysts that absorbed eosin were classified as non-viable, whereas those that did not take up the stain were judged viable (Albalawi & Alanazi, 2023).

Blood sample collection

Following a seven-day treatment period, the mice were euthanized through deep anesthesia induced by a ketamine (100 mg/kg)-xylazine (10 mg/kg) combination. The abdominal cavity was subsequently opened, and blood samples were obtained directly from the cardiac region.

Collecting duodenal tissue

Subsequent to the incision of the abdominal cavity, duodenal tissue was procured and thoroughly rinsed to remove any residual digestive material and then were stored at -80°C for further tests.

Evaluating the electrolyte serum level

These blood samples underwent centrifugation, and the resultant serum was analyzed utilizing diagnostic biochemical kits from Roche, Germany. The analysis was to quantify the serum electrolyte levels of sodium (Na+) and potassium (K+).

Effect on the NF-κB signaling-related genes by qRT-PCR

Total RNA was extracted from the collected duodenal tissue of the tested mice using RNeasy® kits (Qiagen, Hilden, Germany). Subsequently, complementary DNA (cDNA) was synthesized employing the iScript™ cDNA Synthesis Kit (Bio-Rad, USA). Quantitative real-time polymerase chain reaction (qRT-PCR) was performed using SYBR Green PCR master mix sourced from Qiagen (Hilden, Germany). The primers designed to target inflammatory cytokine genes (Tumor Necrosis Factor-alpha (TNF-α), Interleukin-1 (IL-1), IL-10, Nuclear Factor kappa B p65 (NF-κB p65), and Toll-like Receptor 4 (TLR4)) were presented in Table 1. The qRT-PCR procedure included an initial denaturation step at 94°C for 10 minutes, followed by 40 amplification cycles, and concluded with a final extension at 74°C for 5 minutes. The $2^{-\Delta\Delta Ct}$ method was subsequently employed

Table 1. The sequence of primer used in this study

Primer	Sequence (5'–3')	Size (bp)
IL-1	F: AACCTGCTGGTGTGTGACGTTC R: CAGCACGAGGCTTTTTTGTTGT	256
TNF-α	F: TGAACTTCGGGGTGATCGGT R: GGTGGTTTGTGAGTGTGAGGG	182
IL-10	F: GCTGGTTATTGTGCTGTCTC R: GTTCAAAATGCCGATGATCTCT	222
NF-κB p65	F: AGGCAAGGAATAATGCTGTCCTG R: ATCATTCTCTAGTGTCTGGTTGG	209
Toll-like receptor 4 (TLR4)	F: AGCTTTGGTCAGTTGGCTCT R: CAGGATGACACCATTGAAGC	224
β-actin	F: GTGACGTTGACATCCGTAAAGA R: GCCGGACTCATCGTACTCC	245

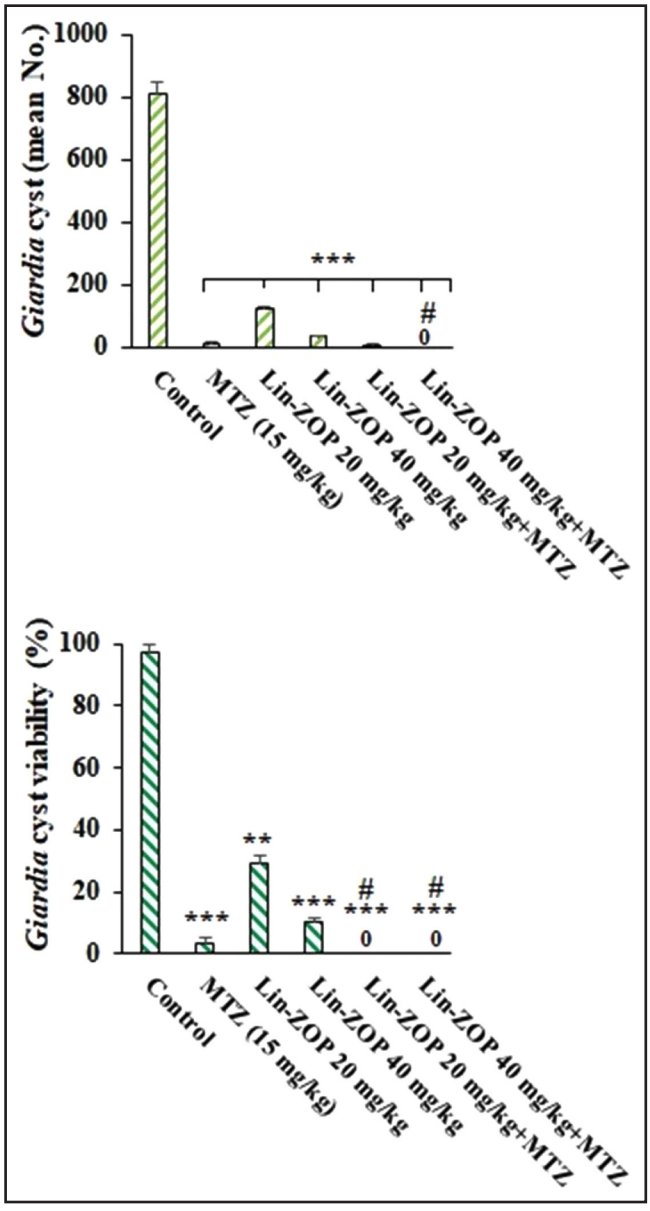

Figure 1. *In vivo* effects of linalool-zinc oxide nanoparticles (Lin-ZOP) and metronidazole (MTZ) on both the number and viability of *Giardia* cysts in infected murine subjects. Statistical significance is indicated by **p<0.01 and ***p<0.001 in comparison to the control group (normal saline), and # p<0.05 when compared to the MTZ treatment.

for data analysis, which was conducted using the Bio-Rad iQ5 Optical System Software (USA) (Masoori *et al*., 2024).

Assessing the impact of toxicity on functional indicators of vital organs

A total of 36 healthy mice were assigned to six separate groups, with each group consisting of six mice. Subsequently, the experimental subjects received oral administration of the designated treatments over a continuous duration of seven days, which included:

i. Administered Normal saline (control group)
ii. Administered with MTZ at 15 mg/kg
iii. Administered with Linalool-ZOP at 20 mg/kg
iv. Administered with Linalool-ZOP at a dosage of 40 mg/kg
v. Administered with MTZ (7.5 mg/kg) + Linalool-ZOP at 20 mg/kg
vi. Administered with MTZ (7.5 mg/kg) + Linalool-ZOP at 40 mg/kg

Following a seven-day treatment period, the mice were euthanized through deep anesthesia induced by a ketamine (100 mg/kg)-xylazine (10 mg/kg) combination. The abdominal cavity was subsequently opened, and blood specimens were obtained directly from the cardiac region. The serum concentrations of renal function biomarkers, namely blood urea nitrogen (BUN) and creatinine (Cr), along with hepatic function indicators, such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), have been identified using the Roche commercial biochemical kits, Germany (Mahmoudvand *et al*., 2019).

Statistical analysis

The resulting data were subjected to analysis utilizing SPSS software version 26.0, specifically employing one-way ANOVA. Additionally, a post hoc test was conducted to evaluate the comparative effects of the tested agents against the control drug. A meaning point of P<0.05 was set to denote statistically significant differences.

RESULTS

Characterizations of Linalool-ZOP

The average diameter of Linalool-ZOP was determined to be 105 nm. Additionally, the zeta potential of Linalool-ZOP was recorded at 28.3 mV. Fourier-transform infrared spectroscopy (FTIR) analysis revealed absorption bands at 3312, 1523, and 712 cm-1, which correspond to the stretching of O-H bonds, the carbonyl group (C=O), and the presence of zinc ions, respectively (Suppl 1). Kinetic release studies indicated that the release rates of ZOP and Linalool exhibited a dose- and time-dependent increase, peaking at 60 minutes before stabilizing (Suppl. 1).

Therapeutic effects of Linalool-ZOP on giardiasis in mice

As illustrated in Figure 1, the average quantity of *G. lamblia* cysts observed was 122.1, 32.4, and 1.36 cysts following a seven-day treatment of *G. lamblia*-infected mice with Lin-ZOP 20 mg/kg, Lin-ZOP 40 mg/kg, and Lin-ZOP 20 mg/kg + MTZ (7.5 mg/kg), respectively; while no *G. lamblia* cysts was observed following a seven-day treatment of *G. lamblia*-infected mice with Lin-ZOP 40 mg/kg + MTZ (7.5 mg/kg). Correspondingly, the viability of *G. lamblia* cysts was diminished by 29.4%, 10.2%, 0%, and 0% after the same treatment regimen. Furthermore, the post hoc analysis indicated that among the evaluated pharmacological agents, Lin-ZOP in combination with MTZ exhibited superior efficacy relative to MTZ ($p<0.05$).

Modulating the serum level of electrolytes

The biochemical analysis revealed a significant reduction in serum Na and K levels in the infected mice ($p < 0.05$). Conversely, the oral administration of Lin-ZOP, particularly in combination with MTZ 7.5 mg/kg, significantly influenced the serum levels of Na and K

in the infected mice ($p < 0.001$) versus mice administrated with normal saline (Figure 2). Furthermore, post hoc analysis indicated that Lin-ZOP, particularly in combination with MTZ 7.5 mg/kg was more effective in modulating serum electrolyte levels than MTZ alone ($p < 0.05$).

Effect on the NF-κB signaling-related genes

The results revealed that in the infected murine subjects, there was a significant increase in the expression levels of the genes *IL-1β, TNF-α, NF-κB p65,* and *TLR4*, accompanied by a decrease in *IL-10* gene expression ($p < 0.05$). In contrast, the administration of Lin-ZOP, particularly in combination with MTZ 7.5 mg/kg independently led to a statistically significant reduction ($p < 0.001$) in the expression of *TNF-α, IL-1, NF-κB p65,* and *TLR4* genes, as well as a marked increase in *IL-10* gene expression (Figures 4 and 5) versus mice administrated with normal saline ($p < 0.001$). Further analysis indicated that among the drugs evaluated, Lin-ZOP, particularly in combination with MTZ 7.5 mg/kg exhibited a more pronounced effect on the modulation of inflammatory cytokines in comparison to MTZ alone, with statistical significance ($p < 0.05$).

Assessing the impact of toxicity on functional indicators of vital organs

The analyses exhibited, while certain parameters showed fluctuations in serum levels of liver (ALT and AST) and kidney (BUN and Cr) function markers, these variations were not statistically significant when versus the control healthy mice (Figure 6).

DISCUSSION

The development of novel and existing pharmaceuticals utilizing nano-sized carriers holds significant potential for addressing various challenges related to the treatment of diseases (Kirtane *et al*., 2021). These challenges include inadequate bioavailability and reducing drug-related toxicities. Additionally, nanocarriers can be employed in the formulation of vaccines, which are critical tools in combating

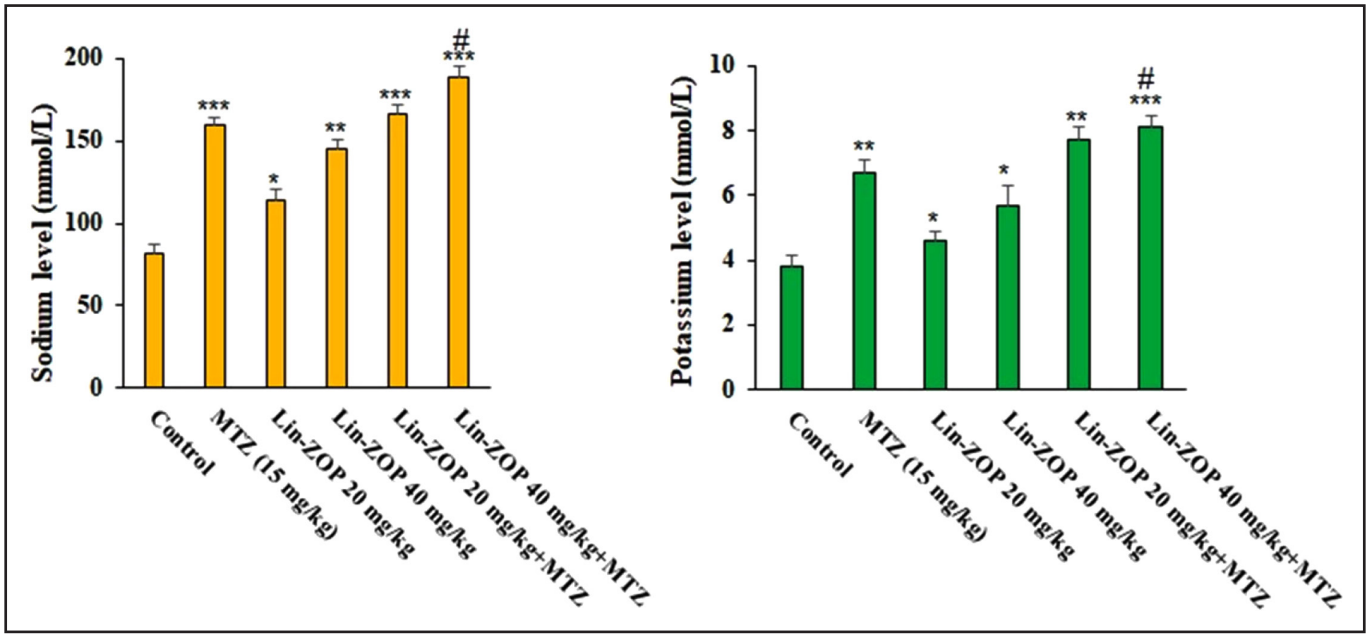

Figure 2. *In vivo* effects of linalool-zinc oxide nanoparticles (Lin-ZOP) and metronidazole (MTZ) on both the sodium and potassium in the Giardia-infected murine subjects. Statistical significance is indicated by * p<0.05, **p<0.01, and ***p<0.001 in comparison to the control group (normal saline), and # p<0.05 when compared to the MTZ treatment.

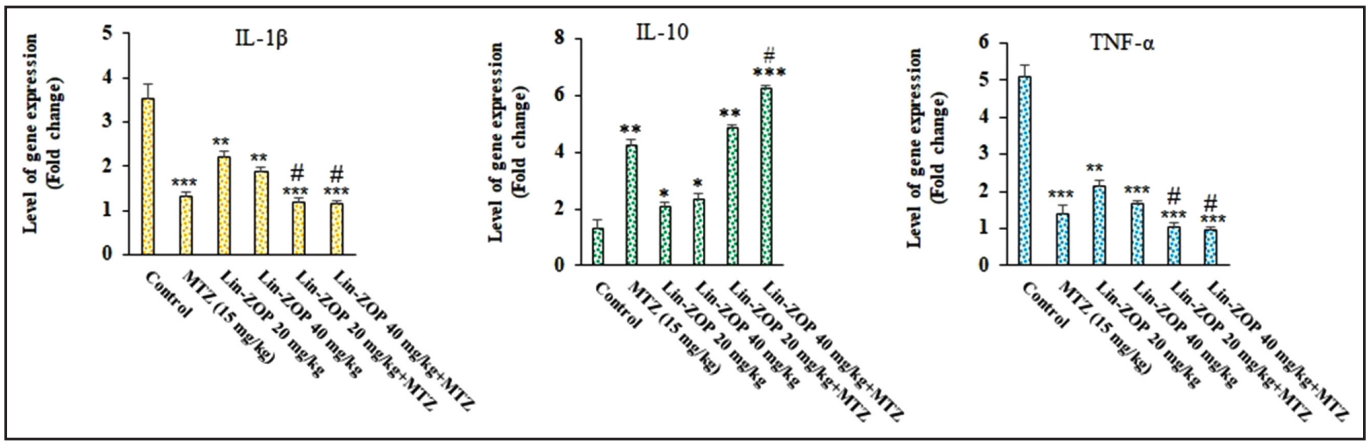

Figure 3. *In vivo* effects of linalool-zinc oxide nanoparticles (Lin-ZOP) and metronidazole (MTZ) on inflammatory genes (Tumor Necrosis Factor-alpha (TNF-α), Interleukin-1 (IL-1β), and IL-10, in the *Giardia*-infected murine subjects. Statistical significance is indicated by * p<0.05, **p<0.01, and ***p<0.001 in comparison to the control group (normal saline), and # p<0.05 when compared to the MTZ treatment.

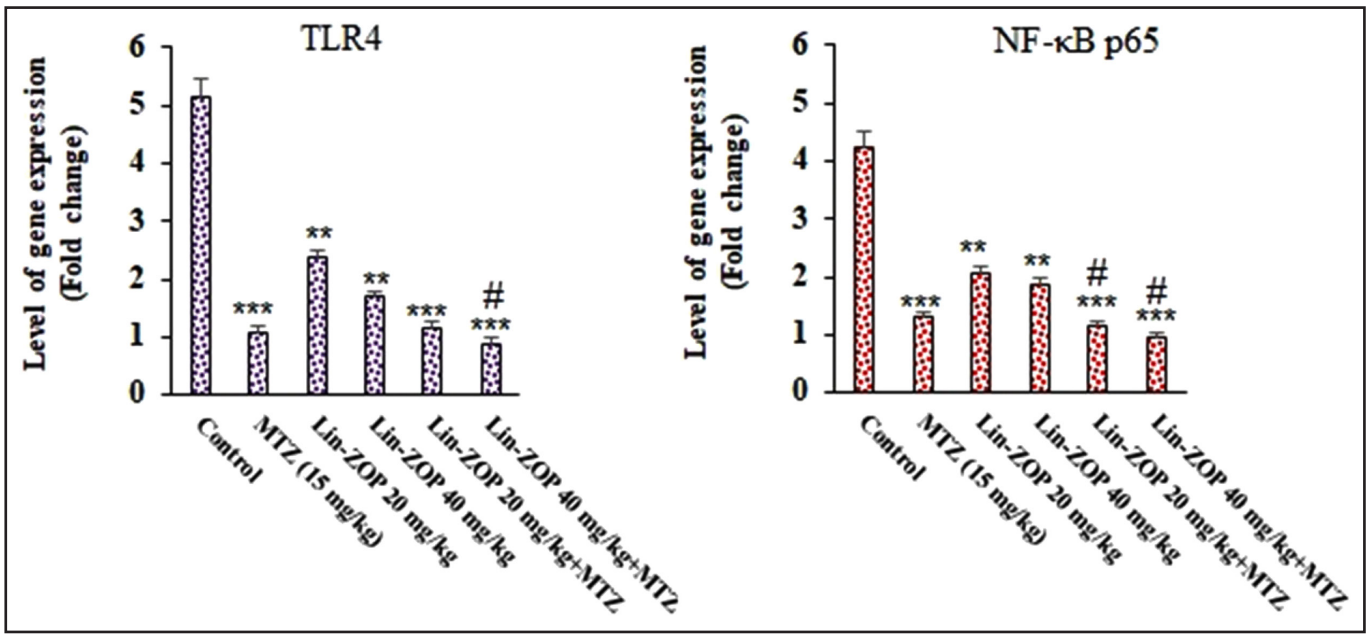

Figure 4. *In vivo* effects of linalool-zinc oxide nanoparticles (Lin-ZOP) and metronidazole (MTZ) on inflammatory genes (Nuclear Factor kappa B p65 (NF-κB p65), and Toll-like Receptor 4 (TLR4)) in the *Giardia*-infected murine subjects. Statistical significance is indicated by * p<0.05, **p<0.01, and ***p<0.001 in comparison to the control group (normal saline), and # p<0.05 when compared to the MTZ treatment.

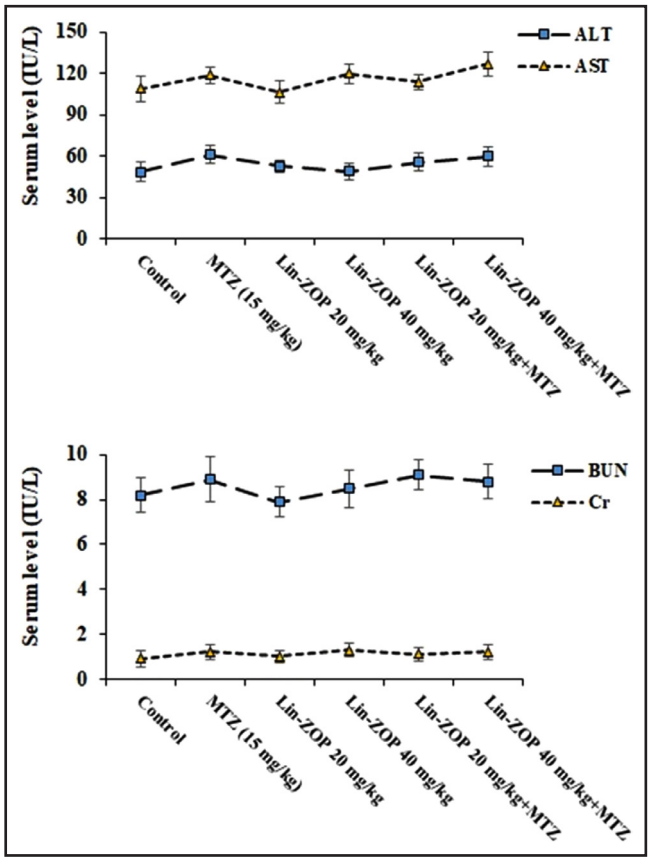

Figure 5. Assessing the impact of toxicity of linalool-zinc oxide nanoparticles (Lin-ZOP) and metronidazole (MTZ) on functional indicators of vital organs of liver (aspartate transaminase (ALT) and alanine transaminase (AST)) and kidney (blood urea nitrogen (BUN) and creatinine (Cr)) of non-infected healthy mice.

infectious diseases (Kirtane *et al*., 2021). The current research seeks to evaluate the *in vivo* therapeutic efficacy of bioconjugated Linalool-ZOP in the treatment of *G. lamblia* infection.

SEM analysis is extensively acknowledged as an essential component in the assessment of morphological characteristics, including the shape and size of synthesized nanoparticles, as well as in elucidating their production methodologies (Xu *et al*., 2023). We found that the average diameter of Linalool-ZOP was determined to be 105 nm. The dimension of nanoparticles is a critical factor influencing their stability and biological functionalities (Gupta *et al*., 2019). Generally, smaller nanoparticles demonstrate enhanced structural integrity and reduced tendency for aggregation (Gupta *et al*., 2019). Furthermore, the application of specific molecular coatings on nanoparticles can augment their size while simultaneously enhancing their biocompatibility and biological characteristics (Xu *et al*., 2023). Our kinetic release studies indicated that the release rates of ZOP and Linalool exhibited a dose- and time-dependent increase, peaking at 60 minutes before stabilizing. Several factors, such as the dimensions, morphology, a propensity to form aggregates in aqueous environments, and stability across diverse pH levels, temperatures, and osmolarities, can significantly impact the release kinetics of nanoparticles (Cheraghipour *et al*., 2023). Additionally, studies have indicated that degradation associated with particle development might also affect the drug release kinetics of nanoscale delivery systems (Rakhshaei *et al*., 2019).

Our *in vivo* results showed that following a seven-day treatment of *G. lamblia*-infected mice with Linalool-ZOP mainly along with MTZ, the number and viability of *G. lamblia* cysts was significantly decreased; indicated the high therapeutic effects of Linalool-ZOP for controlling giardiasis in mice. In relation to the antiparasitic properties of zinc oxide nanoparticles, Delvari *et al.* (2014) provided evidence that these nanoparticles possess significant antileishmanial activity against *Leishmania major* promastigotes, achieving an IC_{50} of 37.8 µg/mL. Whereas, another investigation described that ZNPs had considerable efficacy on mortality of *Toxocara vitulorum* worms,

with concentrations ranging from 0.004% to 0.012% w/v (Dorostkar *et al*., 2017). Additionally, these nanoparticles exhibited notable antiparasitic with an IC_{50} of 3.41 µg/mL on *Plasmodium falciparum* (Najoom *et al*., 2021). Ghasemian Yadegari *et al*. (2023) reported that ZOP exhibited a high efficacy against *Leishmania major* amastigotes (IC_{50}=43.2 µg/mL); while treatment with combination treatment ZOP + glucantime entirely improved cutaneous leishmaniasis lesions in mice. Similarly, Cheraghipour *et al*. (2023) found that ZOP combined with eugenol substantially inhibited the viability of various clinical forms of *T. gondii* (IC_{50}=30 µg/mL). The variations identified in the results may be ascribed to multiple factors, including the specific strain of the parasite, the method employed to produce nanoparticles, and the methodologies utilized for testing. The literature reviews suggest that these nanoparticles possess the capability to inhibit microbes via mechanisms include the disruption of cell membranes, interference with DNA and protein functions, ROS release, and the modulation of the expression of key genes (Gudkov *et al*., 2021).

It has been proven that *Giardia* infection leads to malabsorption of glucose, sodium, and water, alongside a decrease in disaccharidase activity (Khlaf, 2022). This phenomenon may be correlated with a decrease in the absorptive surface area of epithelial cells, which subsequently contributes to the onset of diarrhea (Khlaf, 2022). Here, we found that the oral administration of Linalool-ZOP, particularly in combination with MTZ, significantly modulated the serum levels of Na and K in the infected mice. The findings indicate that Linalool-ZOP has the potential to regulate the symptoms of giardiasis by influencing serum electrolyte levels in infected mice.

Studies reported that *G. lamblia* elicits an inflammatory response marked by the release of IL-1 and TNF-α via several signaling pathways, including NF-κB p65, p38, and extracellular signal-regulated kinase (ERK) pathways (Singer *et al*., 2019). Prior investigations have indicated that the equilibrium and variations in cytokine levels can significantly affect or reflect clinical outcomes (Faria *et al*., 2020). Reviews have indicated that essential oils and their primary constituents can modulate inflammatory responses through TLR pathways and ERK signaling pathways, the inhibition of microglial activation, and the regulation of inflammatory mediator expression (e.g., IL-6, IL-1β, and TNF-α). Additionally, these compounds influence the NF-κB and p38 MAPK pathways, reduce apoptosis, and mitigate oxidative damage (Zhao *et al*., 2022). The administration of Linalool-ZOP, particularly in combination with MTZ 7.5 mg/kg independently led to a statistically substantial lessening in the TNF-α, IL-1, NF-κB p65, and TLR4 genes, as well as a marked increase in IL-10 gene expression versus mice administrated with normal saline; suggesting that these agents effectively manage giardiasis in murine models by exerting anti-inflammatory effects, specifically through the inhibition of the TLRs/NF-κB signaling pathway and the suppression of specific inflammatory cytokines. The assessment of the toxicological characteristics of new pharmacological agents is a critical and necessary process that is generally performed prior to their commercialization (Blomme & Will, 2016). In this context, we examined the safety profile of Linalool-ZOP with respect to liver and kidney function in tested mice. We indicated that, although certain parameters showed variations in serum levels of liver and kidney function markers, these fluctuations were not statistically significant versus to the control group. This finding suggests that Linalool-ZOP does not exert toxic effects on these vital organs in mice. The current investigation revealed encouraging outcomes associated with the application Linalool-ZOP in an animal model of giardiasis. However, there exists a deficiency in comprehensive studies that elucidate the potential mechanisms underlying its effects, as well as a thorough examination of its toxicity profile. If these limitations be addressed, and all pertinent aspects substantiated, zinc nanoparticles may be considered a viable therapeutic agent for the treatment of giardiasis in subsequent stages, pending approval during clinical trials.

CONCLUSION

The present study revealed that the Linalool-ZOP, mainly in combination with MTZ, significantly alleviated *Giardia* infection in murine models by reducing inflammation and rectifying serum electrolyte imbalances. Should additional mechanisms be clarified and subsequent clinical trials involving human subjects produce positive outcomes, these compounds could be considered potential candidates for developing a new therapeutic approach for giardiasis. Furthermore, we advocate for the initiation of human clinical trials and a more comprehensive assessment of the toxicity of Linalool-ZOP in more intricate models.

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Conflict of Interest

The author declares that they have no conflict of interest.

REFERENCES

Albalawi, A.E., Alanazi, A.D., Baharvand, P., Sepahvand, M. & Mahmoudvand, H. (2020). High potency of organic and inorganic nanoparticles to treat cystic echinococcosis: an evidence-based review. *Nanomaterials* **10**: 2538. https://doi.org/10.3390/nano10122538

Albalawi, A.E. & Alanazi, A.D. (2023). Chemical Composition, *in vitro*, and *in vivo* antiparasitic effects of *Curcuma zedoaria* rhizome essential oil against *Giardia lamblia*. *Pharmacognosy Magazine* **19**: 418-426. https://doi.org/10.1177/09731296231166055

Albalawi, A.E., Shater, A.F., Alanazi, A.D. & Almohammed, H.I. (2024). Unveiling of the antileishmanial activities of Linalool loaded zinc oxide nanocomposite through its potent antioxidant and immunomodulatory effects. *Acta Tropica* **252**: 107155. https://doi.org/10.1016/j.actatropica.2024.107155

Aprotosoaie, A.C., Hăncianu, M., Costache, I.I. & Miron, A. (2014). Linalool: a review on a key odorant molecule with valuable biological properties. *Flavour and Fragrance Journal* **29**: 193-219. https://doi.org/10.1002/ffj.3197

Blomme, E.A. & Will, Y. (2016). Toxicology strategies for drug discovery: present and future. *Chemical Research in Toxicology* **29**: 473-504. https://doi.org/10.1021/acs.chemrestox.5b00407

Cheraghipour, K., Khudair Khalaf, A., Moradpour, K., Zivdari, M., Beiranvand, M., Shakib, P., Mahmoudvand, H. & Marzban, A. (2023). Synthesis, characterization, and antiparasitic effects of zinc oxide nanoparticles-eugenol nanosuspension against *Toxoplasma gondii* infection. *Heliyon* **9**: e19295. https://doi.org/10.1016/j.heliyon.2023.e19295

Dorostkar, R., Ghalavand, M., Nazarizadeh, A., Tat, M. & Hashemzadeh, M.S. (2017). Anthelmintic effects of zinc oxide and iron oxide nanoparticles against *Toxocara vitulorum*. *International Nano Letters* **7**: 157-164. https://doi.org/10.1007/s40089-016-0198-3

Faria, C.P., Neves, B.M., Lourenço, Á., Cruz, M.T., Martins, J.D., Silva, A., Pereira, S. & Sousa, M.D.C. (2020). *Giardia lamblia* decreases NF-κB $p65^{RelA}$ protein levels and modulates LPS-induced pro-inflammatory response in macrophages. *Scientific Reports* **10**: 6234. https://doi.org/10.1038/s41598-020-63231-0

Formoso, P., Muzzalupo, R., Tavano, L., De Filpo, G. & Nicoletta, F.P. (2016). Nanotechnology for the environment and medicine. *Mini Reviews in Medicinal Chemistry* **16**: 668-675.

Gudkov, S.V., Burmistrov, D.E., Serov, D.A., Rebezov, M.B., Semenova, A.A. & Lisitsyn, A.B. (2021). A mini review of antibacterial properties of ZnO nanoparticles. *Frontiers in Physics* **9**: 641481. https://doi.org/10.3389/fphy.2021.641481

Gupta, R., Malik, P., Das, N. & Singh, M. (2019). Antioxidant and physicochemical study of *Psidium guajava* prepared zinc oxide nanoparticles. *Journal of Molecular Liquids* **275**: 749-767. https://doi.org/10.1016/j.molliq.2018.11.085

Innes, E.A. (2010). A brief history and overview of *Toxoplasma gondii*. *Zoonoses and Public Health* **57**: 1-7. https://doi.org/10.1111/j.1863-2378.2009.01276.x

Khlaf, W.M.A. (2022). The effect of infection with the parasite *Giardia lamblia* on some physiological, hormonal and biochemical variables. *Journal of Pharmaceutical Negative Results* **13**: 2139-2144.

Kirtane, A.R., Verma, M., Karandikar, P., Furin, J., Langer, R. & Traverso, G. (2021). Nanotechnology approaches for global infectious diseases. *Nature Nanotechnology* **16**: 369-384. https://doi.org/10.1038/s41565-021-00866-8

Laishram, S., Kang, G. & Ajjampur, S.S. (2012). Giardiasis: a review on assemblage distribution and epidemiology in India. *Indian Journal of Gastroenterology* **31**: 3-12. https://doi.org/10.1007/s12664-012-0161-9

Mahmoudvand, H., Pakravanan, M., Aflatoonian, M.R., Khalaf, A.K., Niazi, M., Mirbadie, S.R., Tavakoli Kareshk, A. & Khatami, M. (2019). Efficacy and safety of *Curcuma longa* essential oil to inactivate hydatid cyst protoscoleces. *BMC Complementary and Alternative Medicine* **19**: 187-195. https://doi.org/10.1186/s12906-019-2527-3

Malekifard, F., Tavassoli, M. & Vaziri, K. (2020). *In vitro* assessment antiparasitic effect of selenium and copper nanoparticles on *Giardia deodenalis* Cyst. *Iranian Journal of Parasitology* **15**: 411-417. https://doi.org/10.18502/ijpa.v15i3.4206

Masoori, L., Khalaf, A.K., Ezzatkhah, F., Balaña-Fouce, R. & Mahmoudvand, H. (2024). Promising effects of 1,8 Cineole to control *Giardia lamblia* infection: targeting the inflammation, oxidative stress, and infectivity. *Acta Tropica* **255**: 107201. https://doi.org/10.1016/j.actatropica.2024.107201

Mirhosseini, M. & Firouzabadi, F.B. (2013). Antibacterial activity of zinc oxide nanoparticle suspensions on food borne pathogens. *International Journal of Dairy Technology* **66**: 291-295. https://doi.org/10.1111/1471-0307.12015

Moezzi, A., McDonagh, A.M. & Cortie, M.B. (2012). Zinc oxide particles: synthesis, properties and applications. *Chemical Engineering Journal* **185**: 1-22. https://doi.org/10.1016/j.cej.2012.01.076

Najoom, S., Fozia, F., Ahmad, I., Wahab, A., Ahmad, N., Ullah, R., Gul, A., Bari, A., Khan, M.Y. & Khan, A.A. (2021). Effective antiplasmodial and cytotoxic activities of sSynthesized zinc oxide nanoparticles using *Rhazya stricta* leaf extract. *Evidence-Based Complementary and Alternative Medicine* **2021**: 5586740. https://doi.org/10.1155/2021/5586740

Rakhshaei, R., Namazi, H., Hamishehkar, H., Kafil, H.S. & Salehi, R. (2019). *In situ* synthesized chitosan–gelatin/ZnO nanocomposite scaffold with drug delivery properties: higher antibacterial and lower cytotoxicity effects. *Journal of Applied Polymer Science* **136**: 47590. https://doi.org/10.1002/app.47590

Rohela, G.K., Srinivasulu, Y. & Rathore, M.S. (2019). A review paper on recent trends in bio-nanotechnology: implications and potentials. *Nanoscience & Nanotechnology-Asia* **9**: 12-20. http://doi.org/10.2174/2210681208666171204163015

Singer, S.M., Fink, M.Y. & Angelova, V.V. (2019). Recent insights into innate and adaptive immune responses to Giardia. *Advances in Parasitology* **106**: 171-208. https://doi.org/10.1016/bs.apar.2019.07.004

Vivancos, V., González-Alvarez, I., Bermejo, M. & Gonzalez-Alvarez, M. (2018). Giardiasis: characteristics, pathogenesis and new insights about treatment. *Current Topics in Medicinal Chemistry* **18**: 1287-1303. https://doi.org/10.2174/1568026618666181002095314

Watkins, R., Wu, L., Zhang, C., Davis, R.M. & Xu, B. (2015). Natural product-based nanomedicine: recent advances and issues. *International Journal of Nanomedicine* **10**: 6055-6074. https://doi.org/10.2147/IJN.S92162

Watkins, R.R. & Eckmann, L. (2014). Treatment of giardiasis: current status and future directions. *Current Infectious Disease Reports* **16**: 396. https://doi.org/10.1007/s11908-014-0396-y

Xu, J., Song, M., Fang, Z., Zheng, L., Huang, X. & Liu, K. (2023). Applications and challenges of ultra-small particle size nanoparticles in tumor therapy. *Journal of Controlled Release* **353**: 699-712. https://doi.org/10.1016/j.jconrel.2022.12.028

Yadegari, J.G., Khudair Khalaf, A., Ezzatkhah, F., Shakibaie, M., Mohammadi, H.R. & Mahmoudvand, H. (2023). Antileishmanial, cellular mechanisms, and cytotoxic effects of green synthesized zinc nanoparticles alone and in combined with glucantime against Leishmania major infection. *Biomedicine & Pharmacotherapy* **164**: 114984. https://doi.org/10.1016/j.biopha.2023.114984

Zhao, Y., Yang, Y., Liu, M., Qin, X., Yu, X., Zhao, H., Li, X. & Li, W. (2022). COX-2 is required to mediate crosstalk of ROS-dependent activation of MAPK/NF-κB signaling with pro-inflammatory response and defense-related NO enhancement during challenge of macrophage-like cell line with *Giardia duodenalis*. *PLoS Neglected Tropical Diseases* **16**: e0010402. https://doi.org/10.1371/journal.pntd.0010402