Exp. Theo. NANOTECHNOLOGY 9 (2025) 555-561 https://doi.org/10.56053/9.4.555

Biological activity of nickel oxide nanoparticles against *Leishmania tropica* in vitro

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Received 21/5/2025, *Received in revised form* 17/9/2025, *Accepted* 29/9/2025, *Published* 15/10/2025

The development of Leishmania culture techniques has progressed significantly since the early 20th century, from simple media with limited success in supporting long-term growth to sophisticated formulations enriched with nutrients and vitamins that better mimic the parasite's natural environment. These advances have enhanced the reliability and reproducibility of culture methods, laying a solid foundation for biomedical research and pharmaceutical applications. Currently, anaerobic culture systems enable the maintenance of various Leishmania species in the laboratory under controlled conditions, supporting research into parasite biology, pathogenesis, and drug response. The application of standardized protocols also ensures the consistency of experimental results, particularly in assessing parasite susceptibility to emerging antileishmanial agents. Recent findings highlight the potential of nickel oxide nanoparticles (NiO NPs) as catalytic additives in culture media, as they produce reactive oxygen species (ROS) and disrupt cell membrane integrity, altering parasite metabolism. The incorporation of nickel oxide nanoparticles into conventional media may provide new opportunities to enhance culture efficiency and deepen our understanding of nano-parasite interactions.

Keywords: Nickel Oxide; Nanoparticles; *Leishmania tropica*.

1. INTRODUCTION

Nanomaterials have attracted significant attention in biomedical research due to their unique physical and chemical properties. Nickel oxide nanoparticles (NiO NPs), in particular, stand out for their stability, large surface area, catalytic activity, and biocompatibility [1]. They have been widely investigated for potential applications in drug delivery, antibacterial activity, cancer therapy, and biosensing [2]. Their nanoscale size and tunable surface properties allow them to efficiently interact with biological systems, making them promising candidates for innovative therapeutic strategies [3].

Leishmaniasis, the second most lethal parasitic disease after malaria, remains a major global health challenge. Clinical presentations vary from self-healing cutaneous lesions (CL) to severe visceral involvement (VL), with an estimated 0.7–1 million new cases annually across nearly 100 countries [4]. The disease ranks ninth among infectious diseases in terms of global burden and is recognized as an important opportunistic infection in HIV co-infected individuals [5].

Previous research has shown that culture techniques are essential tools for studying parasitic diseases, even though they are not always employed for routine diagnosis. For protozoa such as Leishmania and trypanosomes, culture has been instrumental in enabling deeper investigations into parasite structure, physiology, and metabolism [6]. The pioneering work of Novy and McNeal in 1904, later that is refined by Nicol in 1908, has introduced the NNN medium as the first successful attempt to cultivate these parasites in vitro [7,8]. Since then, culture systems have greatly advanced parasitology research, supporting better understanding of parasite biology and facilitating the development of control and treatment strategies.

Building on these findings, this study is designed to evaluate the effect of nickel oxide nanoparticles on *Leishmania tropica*. The aim is to explore how NiO NPs influence parasite growth under controlled culture conditions, thereby assessing their potential role as bioactive agents in anti-leishmanial research.

2. EXPERIMENTAL

2.1 Equipment and Instruments

The following laboratory equipment is used in this study:

1. Charles Chamberland's autoclave (1884, France)
2. Incubator (evolved over time; not invented)
1. The concept of a sensitive balance (developed by Joseph Black) (18th century)
2. Filter paper (Karl Friedrich Schönbein) (circa 1860, Germany)
3. Compound light microscope (Zacharias Janssen) (1590, Netherlands)
4. Micropipette (Heinrich Schnittger) (1957, Germany)
5. Orbital vibrator (industrial laboratory invention); no single inventor
6. Carl von Linde's refrigerator (1876, Germany)
7. Robert Bunsen's Bunsen burner (1855, Germany)
8. Micropipette tips related to the development of the micropipette (Schnittger)
9. Test tubes: Commonly used laboratory glassware; No identified inventor
10. Spatula: A basic laboratory tool; inventor unknown
11. Gloves (medical) William Stewart Halsted (1890, USA)
12. Sterile cotton swabs Leo Gerstenzang (1923, USA - modern version)
13. Cork punch: A traditional laboratory tool; source unknown

2.2 Culture Media

The following culture media are used:

1.Roswell Park Memorial Institute 1640 medium

All media are prepared according to the manufacturer's instructions, steam is sterilized at 121 °C for 15 minutes at 15 psi, and is cooled to 40-45 °C before use.

2.3 Chemicals and Reagents

The following chemicals are used:
1. Nanoparticles (nickel oxide nanoparticles) No single inventor is attributed; nanotechnology research has begun in the late 20th century. Nickel oxide nanoparticles have been extensively studied since the 1990.

2.4 Parasite Culture

The Biotechnology Research Center at Al-Nahrain University is used to be the source of the initial strain of *L. tropica* that is obtained. At a temperature of 25 degrees Celsius, the promastigotes of the indigenous Iraqi *Leishmania* strain (MHOM/IQ/1992) are successfully cultivated on RPMI-1640 medium that is supplemented with 10% fetal calf serum.

2.5 Survival tests on protozoa

Protozoan-stage parasites are transferred to RPMI-1640 with 10% fetal bovine serum, pH 7.2. Using equal levels NiO NPs (0.4, 0.2, 0.1, and 0.05 mg/dL), this study has determined the LC50 of C. longa *L. tropica* protozoa in vitro. The concentrations are applied to 10 ml of protozoan culture media in test tubes. We put 8.5 × 10^4 protozoa/ml into each tube with 10 ml of medium and is incubated at 25 °C for 96 hours. Each tube is carefully mixed after each 24-hour interval for negative controls (fatty acid-free cultures used as a baseline).

2.6 Statistical Analysis

The data from the present study is statistically analyzed using Tukey's test to compare the means. A significance level of 0.05 is employed in the test. The analysis of the existing data is performed utilizing SPSS version 22.

3. RESULTS AND DISCUSSION

The current study has found that *Leishmania tropicalis* grow more when it is cultured with of NiO NPs, showing significant differences ($p < 0.05$). NPs have a positive effect as a nutritional medium for the parasite rather than an inhibitory medium. The highest nutrient concentration is recorded at 0.2 mg/dl with different time exposures at 48 hours (67.25 ± 1.11), 72 hours (140.00 ± 2.48), and 96 hours (235.00 ±2.20), as compared with other times of exposure to controls (23.50 ±0.65, 36.75 ± 0.75, 55.00 ± 1.47, ± 1.19, and 0.50 ± 1.19, respectively). At a concentration of 0.4 mg/dL, the number of parasites increases after 96 h (99.50 ± 1.44). Table 1 and Figures 1 and 2 demonstrate that there is no effect on *Leishmania tropicalis* growth at concentrations of 0.1 mg/dl and 0.05 mg/dl.

Table 1 Effect of nickel oxide nanoparticles on *Leishmania tropica.*

Dose	NiO NPs							
	8.5×10Zero dose							
	Time exposure (hour)							
	After 24		After 48		After 72		After 96	
	Mean	Error	Mean	Error	Mean	Error	Mean	Error
Controls	23.50 a A	0.65	36.75^{a} $_{B}$	0.75	55.00^{b} $_{C}$	1.47	74.50^{b} $_{D}$	1.19
0.4mg/dl	27.00 a A	0.41	44.75^{b} $_{B}$	1.03	61.50^{b} $_{C}$	0.65	99.50^{c} $_{D}$	1.44
0.2 mg/dl	40.00 c A	1.47	67.25^{c} $_{B}$	1.11	140.00^{c} $_{C}$	2.48	235.00^{d} $_{D}$	2.20
0.1 mg/dl	33.75 b A	0.75	37.75^{a} $_{A}$	0.48	42.25^{a} $_{B}$	1.03	42.75^{a} $_{B}$	1.25
0.05 mg/dl	40.00 c A	0.41	36.75^{a} $_{A}$	0.75	35.50^{a} $_{A}$	0.65	38.25^{a} $_{A}$	2.66

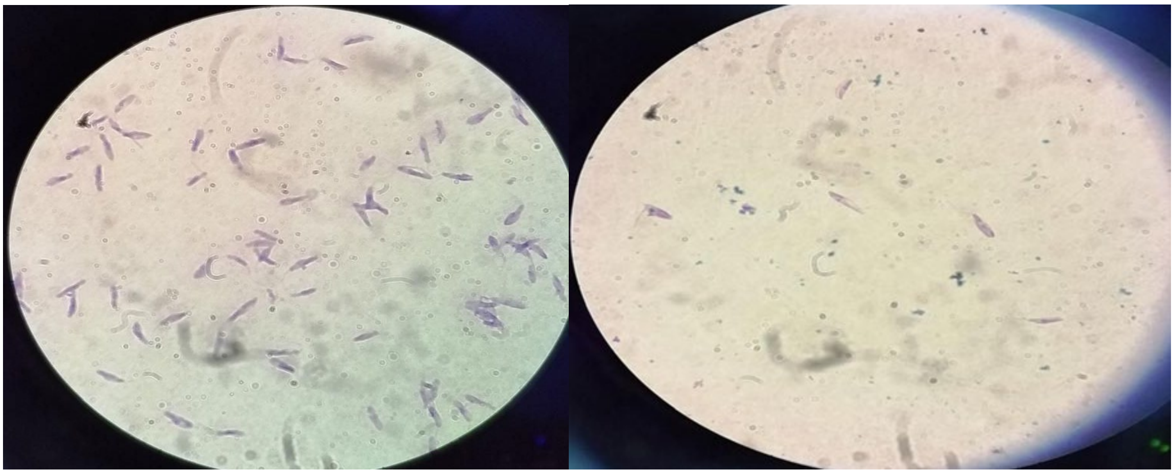

Figure 1 Effect of nickel oxide nanoparticles on the growth of the parasite *Leishmania tropica***.**

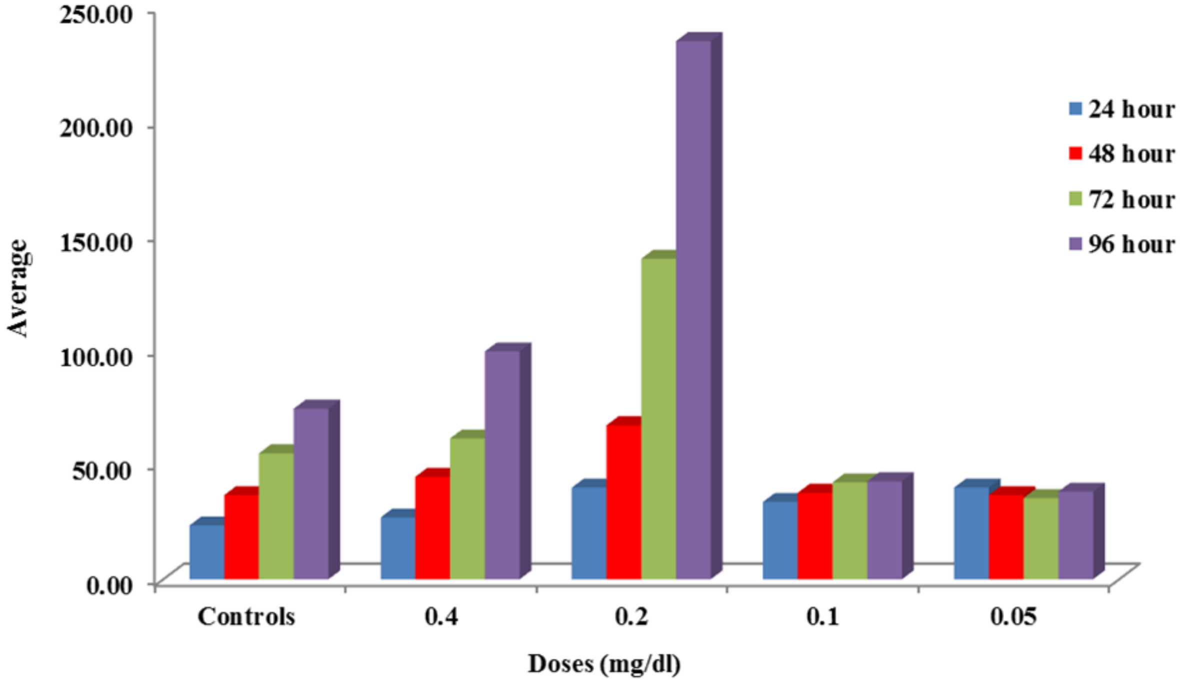

Figure 2 Relationship between concentration and time in the effectiveness of nickel oxide nanoparticles against *Leishmania tropica*.

High catalytic activity, broad surface area, stability, and relative biocompatibility are only a few of the distinctive physical and chemical characteristics that make nickel oxide nanoparticles (NiO NPs) attractive options for biomedical applications [9]. In addition to their possible function as antiparasitics, NiO NPs have a variety of biological properties, including antibacterial, antifungal, and anticancer activity [10]. Numerous investigations have demonstrated that nanoparticles directly impact the oxidative balance and cell membrane integrity of microorganisms, which enhance their antiparasitic efficacy [11,12]. Recent research suggests that NiO NPs can exert an antiparasitic effect on parasites, including *Leishmania*, by inducing oxidative stress and increasing the production of oxygen free radicals (ROS), leading to the induction of apoptosis in the parasites [13].

In the context of *L. tropica* and *L. infantum* promastigotes, research has shown that choosing the appropriate culture medium plays a fundamental role in the success of experiments. The effectiveness of liquid PY media, which contained peptone, yeast extract, and 10% fetal bovine serum (FBS), is compared with that of NNN media, showing that different parasite growth conditions directly correlate with the efficacy of test compounds, including nanoparticles [14].

Because the life cycle of *Leishmania* parasites is complex and requires different culture conditions depending on the parasite stage, maintaining them in the laboratory is a significant challenge. Culture is essential for multiple purposes, including laboratory diagnosis, molecular characterization of clinical isolates, production of antigens and antibodies, drug screening, vaccine testing, and preservation of parasite samples to study host-parasite interactions and epidemiological environments [15].

One of the most important steps in the drug screening process is the identification of compounds with *leishmanicidal* activity. To this end, numerous chemical molecules and nanoparticles are being evaluated for their ability to inhibit or kill the parasite [16]. Reliable protocols are essential for effective drug screening. High-throughput screening (HTS) assays are among the most prominent of these tools, allowing for the rapid testing of thousands of different compounds. These assays are often used to measure the ability of parasites to survive, reproduce, or metabolize after exposure to candidate compounds [17].

Although the use of promastigotes remains common in antileishmanial activity tests, the use of amastigotes is considered more important for evaluating the efficacy of new compounds, including NiO NPs. Numerous attempts have been made to replace fetal bovine serum (FBS) with alternative components such as bovine serum albumin, purine and vitamin blends, elevated concentrations of specific amino acids, hemin, hormones, and even human or animal urine [18-21].

4. CONCLUSIONS

During the early 20th century, *Leishmania* culture witnessed tremendous development. Initially, the use of basic media often has failed to support long-term parasite growth. Over the years, researchers have developed increasingly sophisticated culture media, incorporating a wide range of nutrients and vitamins to more accurately mimic the parasite's natural environment. The culture methods that are developed as a result of these improvements have become more reliable and reproducible, vital for research and pharmaceutical production. Currently, anaerobic culture techniques enable the culture of many *Leishmania* species in a laboratory setting under controlled conditions. This not only facilitates research into the parasite's biology but also its pathogenesis and responses to treatment. Furthermore, standardized protocols should be applied in laboratory procedures to ensure proper cell culture and complementation. This will allow for similar results, particularly regarding parasite sensitivity to new anti-leishmanial chemicals. Recent studies demonstrate that nickel oxide nanoparticles (NiO NPs) can act as a catalyst in the culture medium of *Leishmania tropica*, contributing to the modification of the parasite's metabolic conditions by generating reactive oxygen species (ROS) and affecting cell membrane integrity. Therefore, incorporating NiO NPs into conventional media may open new avenues for improving culture efficiency and understanding the interactions between the parasite and nano-agents.

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