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Pages: 11-28

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Investigation the Effects of Zinc Oxide and Nickel nano- particles on Visceral *Leishmaniasis in-vitro*

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Abstract:

Leishmaniasis is a widespread ailment in the Iraq, encompassing both cutaneous and visceral. The present study was conducted to investigate the anti-leishmanial activity of zinc oxide nanoparticles (ZnO NPs) compared to nickel nanoparticles which both biosynthesized by using clove extract as a reducing agent for particle manufacturing. Bio-based zinc oxide nanoparticles and nickel nanoparticles with an average particle size of less than 100 nanometers (nm) were produced and characterized using by UV–Vis spectroscopy, Scanning electron microscope, FTIR analysis and X-rays diffraction. The antileishmanial activities were investigated through (MTT) 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cell viability assay. After 24 h of exposure with six concentrations nanoparticles (ZnO and Ni NPs), a concentration-dependent growth inhibition was observed. The IC50 values were determined against promastigotes of L. donovani as 59.06 µg/ml for ZnO NPs and 39,55 µg/ml for NiNPs, with observing statistically significant differences ($p < 0.05$). The results indicated that ZnO NPs and NiNPs had noticeable effect on the inhibition ratio and viability of the *L. donovani* promastigotes in a dose-dependent manner under laboratory conditions. recommend study Based on the present results, it has been concluded that ZnO NPs had little effect on *L. donvani* at the same concentrations in vitro. On the other hand, there is a direct destructive effect of nickel on different forms (promastigotes) of *Leishmania donvani* parasite, also, the destruction of parasites increases with concentrations of nickel NPs used, and the best concentration was 160 µg/ml after 24hr.

Aims:

1- Produce synthesized biological nanoparticles including (zinic oxide and nickel) from clove extract.
2- Study the effect of zinc oxide on *Leishmania donavani*.
3- Study the effect of nickel on *Leishmania donavani*.
4-Investigate and compare the impact of these nano- particles on viability of promastigote in MTT assay in *in-vitro* condition.

P-ISSN: 2410-8863
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Pages: 11-28

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Keywords: Zinc oxide and nickel nanoparticles, Leishmaniasis, *L. donovani*

Introduction:

Leishmaniasis is a vector borne infection induced by an obligate protozoon of the genus Leishmania, transmitted by diverse species of sand flies from the genus Phlebotominae in the form of extracellular flagellated promastigotes, which proliferate as intracellular parasites (aflagellate amastigote) within the mononuclear cells of mammalian hosts (Bates, 2007). Visceral leishmaniasis, often known as kala-azar, is a major public health concern worldwide, especially in developing countries. Over 90% of reported cases originate from India, Bangladesh, Nepal, Sudan, and Brazil (Mansueto *et al*., 2014). Nanobiotechnology is a branch of nanotechnology that enables the production of nanoparticles (NPs) from biological origins for various applications with less hazardous effects. Numerous biological materials exist in nature, such as plants, algae, fungi, yeasts, bacteria, and viruses, all of which may be used for nanoparticle production (Ahmad *et al*., 2003) (Al-Difaie, 2024). Nanosystems are dynamic drug delivery mechanisms and pharmaceutical advancements at the nanometre scale (1–100 nanometres) used for diagnostics, therapy, prevention, or theranostics (Najahi-Missaoui *et al* ., 2020). The mechanism in which nanoparticles interact with bacterial cells is that the metal oxides have a positive charge while the bacterial cells have a negative charge, resultant electromagnetic gravitation between the nanoparticle surfaces and bacteria (Farman and Abood, 2024). They may safeguard pharmaceuticals from degradation, modify pharmacokinetics and bio distribution, and mitigate toxicity. A nanocarrier refers to a system for medication delivery. A main mechanism by which ZnO-NPs display cytotoxicity is the formation of reactive oxygen species (ROS), which alters the redox balance of parasites and promotes cell death (Kumari *et al*., 2017). ZnO nanoparticles are acknowledged as safe and superior nanomaterials due to their biocompatibility, biosafety, and nontoxicity, equivalent to carbon nanotubes, graphene, and gold, and have been widely used in many domains (Ogunyemi *et al*., 2016). The nanoparticles of Nickel have attracted considerable attention in recent studies because to their remarkable chemical stability, superior conductivity, and advantageous optical, electrical, and magnetic properties (Anand *et al* ., 2020). nickel oxide nanoparticles (NiO NPs) are a p-type semiconductor metal oxide distinguished by a broad band-gap range of 3.6 to 4.0 eV. (Vasudeo and Pramod, 2016). nickel nanoparticles have many uses in catalysis, fuel cell electrodes, solar energy absorption, magnetic recording media, magnetic fluids, permanent magnets, and antibacterial activities (Sasi *et al*., 2002). This study examines the anti-leishmanicidal efficacy of ZnO nanoparticles on growth rates and viabilities of L. donovani promastigotes, in comparison with nickel nanoparticles under *in -vitro* circumstances**.**

P-ISSN: 2410-8863
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https://djvs.uodiyala.edu.iq
Pages: 11-28

Diyala Journal for Veterinary Sciences
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Materials and Methods:

Ethical approve:

This study was approved for the animal care at the College of Veterinary Medicine, University of Al-Qadisiyah, under the No. 5129 dated 28/11/2024.

Synthesis of Zinc Oxide and Nickel Nanoparticles by Green Method using clove extract:

Plant Extract:

This study included the acquisition of clove plants from local markets in Al-Diwaniyah.

Preparation of Alcohol extract of clove:

According to the Soxhlet method (Adegbanke and Bada, 2024) with some modification dried clove flower buds were rinsed with distilled water to eliminate contaminants. The cleaned buds were dried then ground into a fine powder using a grinder. Subsequently, the Soxhlet extraction was performed on each sample in (20g) of the ground sample were put in a thimble-shaped filter cloth and inserted into the Soxhlet extractor thimble. The apparatus was then built, and add ethanol 99% (about 3:1 v/w of the sample) was introduced into the reservoir flask prior to being positioned on a heating mantle. Upon heating, the condensed vapors of the solvent interacted with the sample powder, facilitating the dissolution of the soluble component of the powder into the solvent for extraction. Once the solvent surface exceeded the siphon's maximum elevation, the solvent carrying the extract was syphoned back into the flask. The sample was subjected to reflux for 8 hours at a controlled temperature of 55 - 60℃. The heating was terminated at the completion of the reflux time. The solvent was evaporated and collect the extract, as seen in the figure (1)

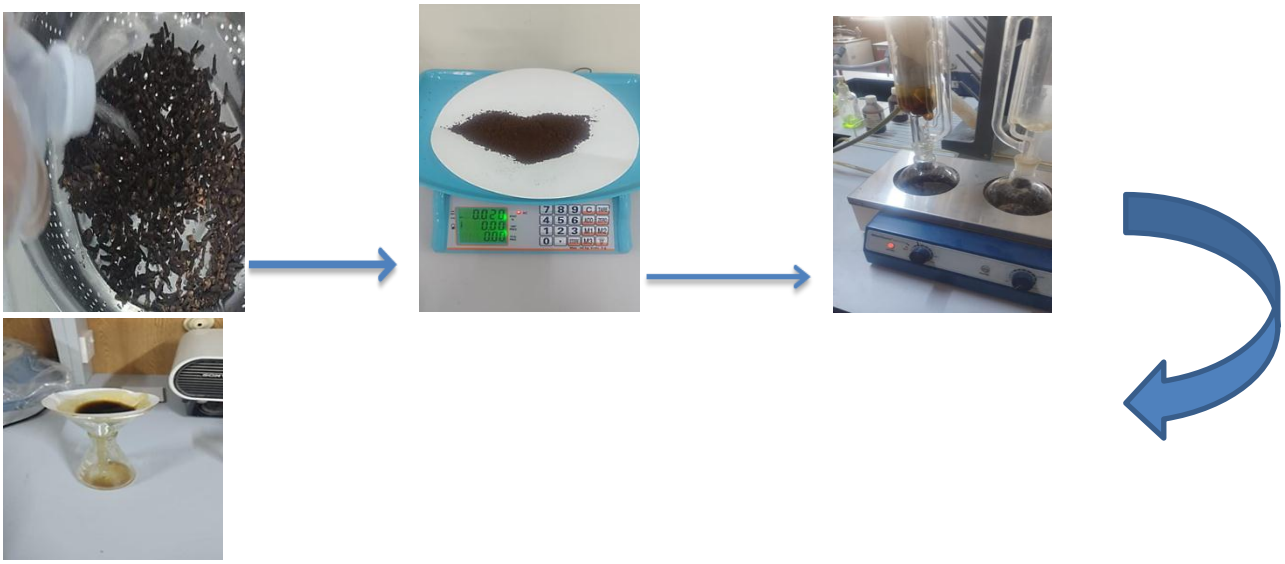

Figure (1) The extract of clove from the Soxhlet apparatus

P-ISSN: 2410-8863
E-ISSN:2958-6178
https://djvs.uodiyala.edu.iq
Pages: 11-28

Diyala Journal for Veterinary Sciences
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The green synthesis of zinc oxide nanoparticles from the clove extract:

The preparation was conducted in accordance with modified method outlined by (Islam *et al*., 2024). The production of Zinc Oxide nanoparticles was procedure, using100 ml of ethanol 99 % add to extract of the clove (20g) and take (20 ml) of mixture and add to 250 mL of ethanol 99 % in beaker placed on a magnetic stirrer Figure (2). The temperature was set to 65 °C. Add 100 ml of ethanol 99% mixing with (10g) zinc acetate and dropping extract to beaker until the reaction mixture, it was allowed to cool to ambient temperature and stand for 4 hours, followed by centrifugation at (7000) rpm for 5 minutes. The supernatant was discarded, and the precipitate was subjected to two to three washings with deionised water, followed by ethanol. The resultant residue was dried at 130 °C for 3 hours to eliminate moisture. The sample was subsequently ground into fine powders and calcined for 4 hours at 250 °C to eliminate any impurities.

Figure (2) :Zinc oxide after adds clove extract on magnetic stirrer apparatus

The green synthesis of Nickel Nanoparticles from the clove extract:

A green synthesis method was employed for the production of biogenic nickel nanoparticles with minor modifications (Balto *et al* ., 2023). Add (100) mL of ethanol 99 % to extract of the clove (10g) and the mixture add to (100) mL of ethanol 99% combined with (20g) nickel nitrate 6- hydrate. and put in beaker placed on a magnetic stirrer precursor and heated at 70–80 °C for 4 hours to facilitate a full oxidation and reduction process. Subsequent to extensive agitation. The production of biogenic nickel pellets was validated by the change in colour bright green to dark green figure (3). Subsequently, the precipitates were collected from the bottom of the flask following centrifugation at 15,000 g for 30 minutes. Following centrifugation, the pellets were retrieved, and the transparent supernatant was discarded. The acquired pellets were dissolved in deionized water and meticulously cleaned many times to eliminate impurities. The pellets were then dried overnight in an oven at 100 °C for 2h and calcined at 300 °C for 1h to optimal crystallization. The calcinated nickel nanoparticles were pulverized using a grinder and kept in glass vials for further examination.

P-ISSN: 2410-8863
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https://djvs.uodiyala.edu.iq
Pages: 11-28

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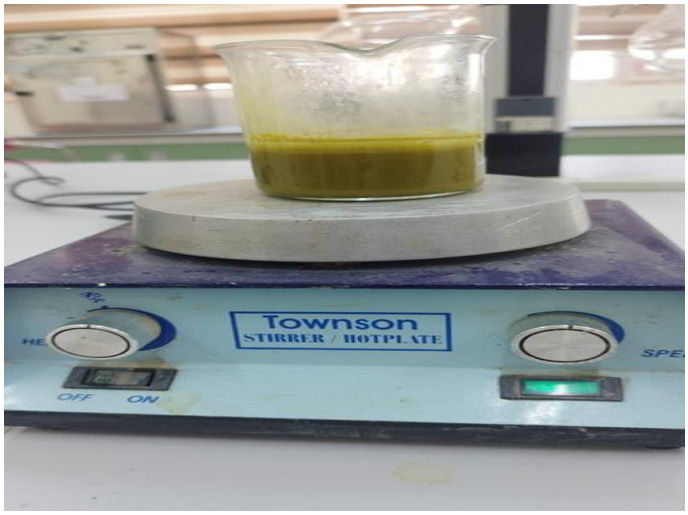

Figure (3): nickel after add clove extract on magnetic stirrer apparatus

Parasite culture:

The molecularly diagnosed parasite was obtained from Biotechnology Research Center, Al-Nahrain University, then cultured using RPMI-1640 medium prepared from American Bio innovations (USA) in the form of a package of 500 ml.

Parasite survival assay:

leishmania parasite (101 parasite ml - 10 × 1 to) were prepared then placed in a 96-well plate with a flat bottom and where the dishes were covered with sterile cling film and gently turned over. They were incubated in an incubator have CO2 at 37°C for 24 hours. After incubation, the medium was removed, after incubation, (100 µl) of parasite suspension was added to each well. The concentration prepared zinc oxide and nickel nanoparticles from the clove extract were added (5, 10, 20, 40, 80, 160 microliter). The plate was incubated for 24 hours at 37°C. When (10 µl) of MTT solution was introduced to each plate at a concentration of 0.45 mg/ml. The plate was incubated for four hours at 37 degrees Celsius. (100µl) of the solubility solution was added to each plate in order to dissolving to the Formazan crystals and then incubated for 5 min and the absorbance of the sample was read at a wavelength of 570 nm using an ELISA device at a wavelength of 575 nm (Bio-rad Germany). Statistical analysis of optical density (OD) readings to calculate IC50 according to the following equation (Denny and Smith, 2004).

***Viability (%) =optical density of sample/ optical density of control x100**

Statistical analysis

The data of the present study was presented as mean of viability percentage and standard error, and the statistical analysis was conducted by using SPSS program version 27. The one-way ANOVA

P-ISSN: 2410-8863
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https://djvs.uodiyala.edu.iq
Pages: 11-28

Diyala Journal for Veterinary Sciences
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followed by least significant difference (LSD) test was applied, and a P value of less than 0.05 was deemed significant. (Daniel, 2009)

Results:

Results and discussion:

Examination and characterization of Green- of Nickel and zinc:

1-UV visible spectroscopy analysis*:*

The absorption spectra of the prepared nickel nanoparticles and prepared zinc oxide nanoparticles dispersed in BPS is shown in Figure (4). The absorption (SPR) peak is obtained in the range at 254 nm and 376 nm respectively. In another study by UV–VIS analysis with absorption peaks of nickel nanoparticles ranging from 230 to 330 nm. (Soliman *et al* ., 2020). in another study the bio reduction of zinc oxide nanoparticles from alcoholic Zno solution, the reactions were studied under UV-vis spectroscopy absorption peaks 294–319 nm (Mohan *et al* ., 2023) found that SPR bands are affected by the size, shape, morphology, composition, and dielectric environment of the produced nanoparticles.

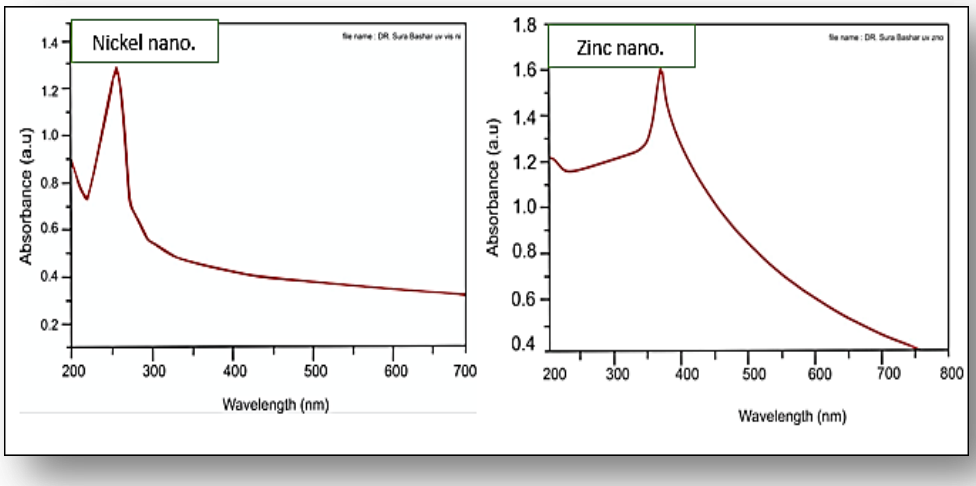

Figure (4): The UV-visible spectrum graph of prepared nickel and zinc nanoparticle

2-Scanning electron microscope:

displays the SEM picture of the synthesized colloidal solution containing nickel and zinc nanoparticles show in Figure (5). The synthesized nickel nanoparticles have a spherical or almost spherical shape. Certain particles may exhibit minor variations from an ideal spherical form, with the diameter of the nanoparticles measured at roughly rate 61.12±2.36 nm for a sample of 100 particles**.** The zinc nanoparticles also exhibit a spherical or nearly spherical morphology, similar to the nickel

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https://djvs.uodiyala.edu.iq
Pages: 11-28

Diyala Journal for Veterinary Sciences
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https://doi.org/10.71375/djvs.2025.03202

nanoparticles. and the diameter of the nanoparticles is found to be approximately rate 68.42±1.74 nm for 100 particles. While (Mohamed *et al*., 2023). used same concentration of ZnO nps , the size of the particles manufactured for the ranged from 56.85 nm to 65.6 nm). Showed result in various studies near in range 65.3nm and (Goel *et al* ., 2020) (Jennifer *et al*., 2023) have same concentration of nickel nps showed 70.8nm.

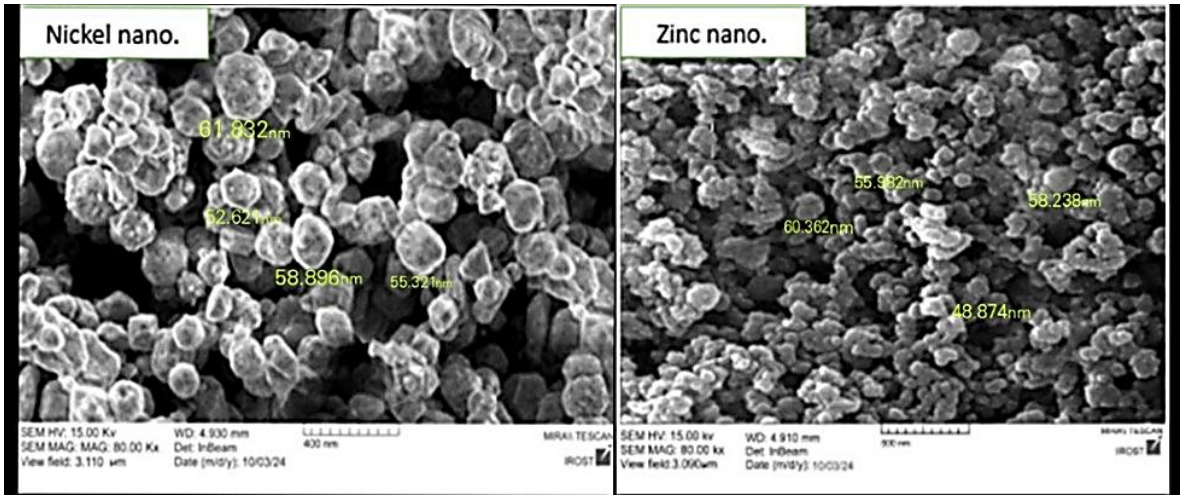

Figure (5): SEM image for prepared nickel and zinc nanoparticles

3- FTIR analysis:

The Fourier transform infrared spectra (FTIR) were analysed in the range of 0–4000 cm-1 using the KBr pellet technique to identify organic, inorganic, and biomolecular residues, as well as nanoparticle formation, potentially introduced by the substance that reduces on the outermost layer. Nickel and zinc nanoparticles the absorption bands for synthesized nickel nanoparticles at 3695, 3624, 3520, 2510, 1890, 1736, 1649, 1583, 1156, 1010, 760, and 510 M-1.

(O-H) Stretching: The peak around 3695 cm-1 is likely due to (O-H) stretching vibrations, which could be from hydroxyl groups in the clove extract or adsorbed water. (C-H) Stretching: The peaks around 3520 cm−1 and 2510 cm−1 can be attributed to (C-H) stretching vibrations, commonly found in organic compounds like those present in clove. (C=O) Stretching: The peaks around 1600-1800 cm−1 (e.g., 1600 cm−1, 1649 cm−1, 1583 cm−1) might indicate the presence of carbonyl groups (C=O), which could be from organic compounds in the clove. (C-O) Stretching: The peaks around 1156 cm−1 and 1010 cm−1 could be due to (C-O) stretching vibrations, which are common in organic molecules. The absorption bands for synthesized zinc nanoparticles recorded at 3435, 2917, 2365, 1633, 1032, 880, and 700 m−1. Figure (6)

(O-H) Stretching: The peak around 3435 cm−1is likely due to O-H stretching vibrations, which could be from hydroxyl groups in the clove extract or adsorbed water. (C-H) Stretching: The peak around 2917 cm−1 can be attributed to (C-H) stretching vibrations, commonly found in organic compounds like those present in clove. (C=O) Stretching: If there is a peak around 1633 cm−1, it might indicate the presence of carbonyl groups (C=O), which could be from organic compounds in

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https://djvs.uodiyala.edu.iq
Pages: 11-28

Diyala Journal for Veterinary Sciences
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the clove. (C-O) Stretching: The peak around 1032 cm−1could be due to C-O stretching vibrations, which are common in organic molecules. The peak around 1032 cm−1could be due to C-O stretching vibrations, which are common in organic molecules have closed results of zinc NPs (Ullah *et al*., S2024). The peaks around 1156 cm−1 and 1010 cm−1 could be due to (C-O) stretching vibrations, which are common in organic molecules close to nickel NPs (Karpagavinayagam *et al*., 2022).

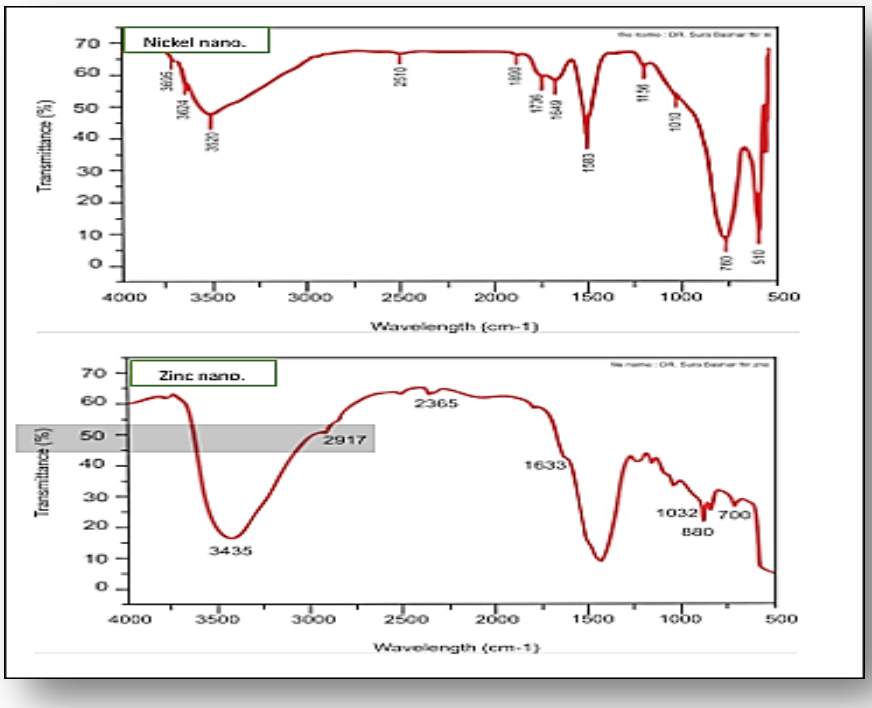

Figure (6): FTIR spectra for prepared nickel and zinc nanoparticles

4- XRD analysis:

X-ray diffraction investigations were conducted on synthesised nickel and zinc nanoparticles to determine their crystallinity It may be identified as the cubic shap of zinc and nickel structure with different quantities and average particle size. The subsequent figure (7) illustrates the XRD pattern of the synthesised nickel nanoparticles. The Bragg reflections for nickel nanoparticles are seen at 2θ = 24.23° (109), 33.92° (210), 24.08° (230), 66.73° (315), and 69.08° (212). (Youssry *et al*., 2023) have closed results. The diffraction peaks for synthesised zinc nanoparticles are seen at 2θ = 32.14° (102), 34.36° (002), 36.01° (103), 47.14° (101), 56.12° (113), 64.02° (114), 66.32° (200), 67.75° (112), and 69.21° (103). mention by (Phogat et al.,2024) have closed results.). The chemical compounds in plant extracts vary depending on the sampling area, and the extraction method also affects these compounds, and this is confirmed by (Zahra and Ahmad, 2020).

P-ISSN: 2410-8863
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https://djvs.uodiyala.edu.iq
Pages: 11-28

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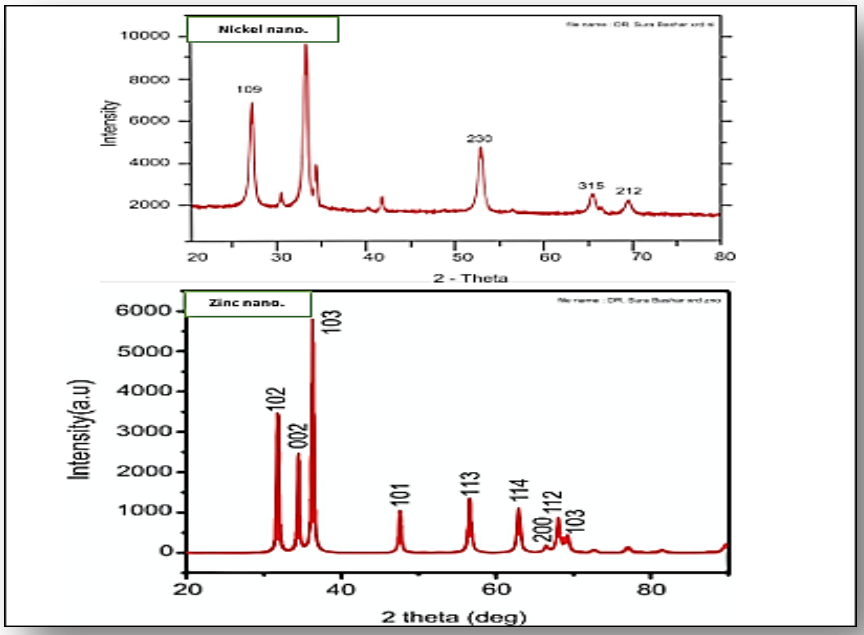

Figure (7): XRD pattern of prepared nickel and zinc nanoparticles

Cytotoxic effect of ZnO NPs and Nickel nanoparticles on L. donvani promastigotes by colorimetric assay (MTT):

The outcomes indicated that the maximum concentration (160 µg/ml) of ZnO nanoparticles after 24 hrous exhibited the maximum cytotoxic effect (9.01±0.26%), whereas the viability of promastigotes at the similar concentration and duration for nickel nanoparticles was (7.91±2.18%), which was lower than that of ZnO nanoparticles. This demonstrates that the optimal concentrations of nickel nanoparticles effectively eradicated a significant number of the viability of promastigotes parasites. Conversely, the minimal concentrations of ZnO NPs and nickel NPs (5 mg/ml) after 24 hours exhibited enhanced viability rates of 98.54±1.46% and 95.24±2.90%, respectively (Table-1), indicating a reduced lethality towards parasites at this concentration.

P-ISSN: 2410-8863
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https://djvs.uodiyala.edu.iq
Pages: 11-28

Diyala Journal for Veterinary Sciences
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Table (1) the Percentages of viability % after incubation of Leishmania donovani promastigotes with zinc oxide and nickel nanoparticles. Data are expressed as the mean ± SD (P<0.05)

Concentration mg/ml	Percentage of viability % (mean ± SE)	
	zinc oxide Nps	Nickel Nps
5	98.54±1.46 a	95.24±2.90 a
10	84.52±11.16 ab	83.71±7.03a
20	75.76± 5.33b	41.53±7.80b
40	29.75± 3.60 c	20.54±2.32b
80	23.6±5.14 cd	13.7±0.94c
160	9.01±0.26 c	7.91±2.18c
LSD (p< 0.05)	17.56	14.34

*Mean the difference between the letters is the significant difference between the concentrations**.**

The calculated IC50s of ZnO NPs and nickel NPs on the viability of L. donovani promastigotes:

The IC50 was computed based on the MTT assay findings to identify the concentrations that had the greatest impact on L. donovani promastigotes' viability. The IC50 values for ZnO nanoparticles were 59.06 mg/ml and for nickel nanoparticles were 39.55 µg/ml, respectively, indicating a important difference ($p < 0.05$) between them, as presented in Table 2.

P-ISSN: 2410-8863
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https://djvs.uodiyala.edu.iq
Pages: 11-28

Diyala Journal for Veterinary Sciences
Vol. 3 No.2 June (2025)

https://doi.org/10.71375/djvs.2025.03202

(Table 2) The calculated IC50 of ZnO NPs and nickel NPs on L. donovani promastigotes viability by the rate of growth assessment:

Concentration (µg/ml)	ZnO NPs	nickel NPs
5	98.54±1.46 a	95.24±2.90 a
10	84.52±11.16 ab	83.71±7.03ab
20	75.76± 5.33b	41.53±7.80ac
40	29.75± 3.60 c	20.54±2.32b
80	23.6±5.14 cd	13.7±0.94c
160	9.01±0.26 c	7.91±2.18cb
IC50	59.06 µg /ml	39.55 µg /ml

The protozoan flagellates of twenty different species and subspecies belonging to the genus Leishmania, family Trypanosomatidae, and order Kinetoplastida are responsible for the manifestation of leishmaniasis, which is a disease transmitted by vectors. The yearly occurrence of newly identified cases is thought to range between 1.5 and 2 million, with 12 million individuals now affected globally. (Mansueto *et al* ., 2014) The Leishmania stage of life starts when parasites are delivered to their host by an attack of an infectious female Sands flies. Sands flies' vectors become infected by ingesting blood from the infected vertebrate vector (Dinesh *et al* ., 2000). Visceral leishmaniasis (VL) exhibits a death rate of 100% if left untreated (Zijlstra *et al* ., 2003). The outcomes of the in vitro cytotoxicity research were acquired during a 24-hour incubation of the sample (Hussein et al ., 2021). A linear correlation exists between the visual density and cell count during incubation durations of 2, 4, 6, or 24 hours, with 10 µl of MTT (15 mg/ml) applied to 100 µl of media. Nanoparticles possess potential in the quest for novel agents targeting various bacteria. In the current work, we investigated the leishmanicidal activity of ZnO nanoparticles on L. donovani.
This study employed a significant viability assay (MTT) at various concentrations (5, 10, 20, 40, 80, 160 µg/ml) to assess the cytotoxic impact of ZnO nanoparticles on L. donovani promastigotes. The growth-inhibitory impact of six concentrations (5, 10, 20, 40, 80, and 160 µg/ml) of ZnO nanoparticles on the promastigotes was assessed after 24 hours. The outcomes indicated the cell growthly inhibition impact is reliant on dose, with the 160 µg/ml concentration of ZnO NPs exhibiting a highest death rate after 24 hours of incubation, while the 5 µg/ml concentration demonstrated the lowest mortality rate for the same duration. Statistically significant differences ($P<0.05$) were observed in development of parasites introduced to various concentrations of ZnO nanoparticles (5, 10, 20, 40, 80, and 160 µg/ml) after 24 hours, with respective growth rates of

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98.54±1.46%, 84.52±11.16%, 75.76±5.33%, 29.75±3.60%, 23.6±5.14%, 23.6±5.14%, and 9.01±0.26% cell/ml. The findings indicated that the vitality of L. donovani promastigotes diminished significantly upon exposure to elevated concentrations of ZnO nanoparticles. These findings align with previous studies that proved the efficacy of ZnO nanoparticles in vitro against Leishmania species - (Enad and Zghair, 2016), (Delavari *et al*.,2014). Additionally, another study indicates that ZnO nanoparticles exert several impacts on a wide array of microorganisms, especially fungal and bacterial and parasites, and viruses (Premanathan *et al*.,2011). The IC50 result of zinc nanoparticles (59.06 µg/ml) supports the hypothesis that nanoparticles bind to various regions of the promastigote membrane, eliminate proteins that compose the membrane, and subsequently inhibit access to phagocytes, resulting in the inactivation and impairment of parasites. In another study examined the IC50 value for leishmania parasites, indicating that the IC50 of ZnO nanoparticles on the Iranian strain of L. major promastigotes was detected at 37.8 µg/ml after 24 hours. This number is inferior to those in this study this might be attributable to variations in parasite strains, discrepancies in nanoparticle manufacturing companies, and differences in ambient and in in-*vitro* settings. The aforementioned results demonstrated the significant efficacy of ZnO nanoparticles against parasites, attributing this effectiveness to the nanoparticles' capacity to generate reactive oxygen species (ROS) that can induce alterations in macromolecules such as proteins, lipids, and nucleic acids, a phenomenon recognised as oxidation a stress. Oxygen-derived free radicals are ephemeral, uneven and affect the viability, integrity, and health of bacterial and eukaryotic cells. Consequently, resulting in cellular apoptosis (HU,2000) and (Palmieri and Sblendorio, 2007). Reactive oxygen species (ROS) induce protein oxidation and lipid peroxidation, compromising cell membrane stiffness by altering liquidity and permeability, penetration, thereby disrupting the transmission of ions and limiting metabolism (Catalá, 2009) (Meaad and Ban, 2017).

The MTT technique was employed to evaluate the impact of various nanoparticle concentrations (5, 10, 20, 40, 80, 160 µg/ml) on the promastigotes of L. donovani. Ni-NPs exhibited dependent on dosage cytotoxicity, with death rates of 95.24±2.90%, 41.53±7.80%, 83.71±7.03%, 20.54±2.32%, 13.7±0.94%, and 7.91±2.18% against promastigotes at varying concentrations of Ni-NPs.The results indicated that the developmental inhibitory impact is dependent on dose, with a concentration of 160 µg/ml of Ni-NPs exhibiting a larger mortality rate after 24 hours of incubation, while a concentration of 5 µg/ml demonstrated a reduced mortality rate after the same duration. The statistically significant IC50 ($P<0.05$) for promastigotes was determined to be 39.55 µg/mL.Previous research indicated that elevated quantities of nickel nanoparticles had significant antioxidant activity. Ni-NPs exhibited dependent on dosage cytotoxic versus both the amastigote and promastigote forms of Leishmania tropica, resulting in a significant death rate (Sana *et al* ., 2021). Ni-NPs exhibit intrinsic cytotoxic characteristics that can provoke oxidative stress in Leishmania tropica, resulting in the suppression of their proliferation. The IC50 identified in this investigation for promastigotes was 39.55 µg/ml. In proximity to research, the NiONPs shown significant anti-leishmanial efficacy against L. tropica promastigotes (IC50: 25.62 µg/ml). Our additional sample was classified under general causing cytotoxicity exhibiting an IC50 value of 43.73 µg/ml, which aligns with prior

P-ISSN: 2410-8863
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https://djvs.uodiyala.edu.iq
Pages: 11-28

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Vol. 3 No.2 June (2025)
https://doi.org/10.71375/djvs.2025.03202

findings of (Saleem *et al* ., 2019) and (Iqbal *et al* ., 2019). Research suggests that the active chemicals in the plant extracts engage with the nickel salts to diminish or form combinations with the metallic ion (Rahdar *et a*l ., 2015). The influence of nickel is evidenced by the production of reactive oxygen species upon light exposure and the direct interaction of nickel oxide nanoparticles with microbial cells, resulting in plasma membrane disruption and the liberation of antibiotic ions, predominantly nickel ions. (Adinaveen *et al* ., 2019, Sabouri *et al* ., 2020, - Gebretinsae *et al* ., 2021). The researchers found that the primary cause of zinc oxide nanoparticles' antimicrobial activity is stress brought on by the production of reactive oxygen species, which also damages the cell membrane's integrity and damages the cell's proteins, mitochondria, and DNA, ultimately causing cell death (Berhe and Gebreslassie , 2023). In another study effect of ZnO-NPS was evaluated for its antimicrobial properties and high antibacterial activity against S. aureus, P. aeroginesa, and E. coli (Kadhim, 2023).

The study results showed that the effect of nano nickle was significantly higher than nano zinc oxide. The reason for the superiority is due to the antiparasitic effect through the generation of reactive oxygen species (ROS) resulting in oxidative stress and cell death. Studies indicate that nano nickel causes cellular damage but at high concentrations. On the other hand, nano zinc and nickel is cheaper and easier to produce, which makes it amenable to development in antiparasitic treatments.

Conclusion:

The current data indicate that nickel had little influence on L. donovani at comparable doses in vitro. Conversely, ZnO nanoparticles have a direct damaging impact on the promastigote form of the L. donovani parasite.

Recommendations:

1. Study the effect of zinc oxide and nickel on other immune and biochemical parameters.
2. Study the effect of zinc oxide and nickel on the visceral leishmaniasis parasite in vivo.
3- Conduct future studies on the produced zinc oxide and nickel nanoparticles and apply them in other environmental, agricultural and medical fields.

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https://djvs.uodiyala.edu.iq
Pages: 11-28

Diyala Journal for Veterinary Sciences
Vol. 3 No.2 June (2025)

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