

RESEARCH PAPER

Evaluating the Role of a Biosynthesized Nanocomposite Using the *Ganoderma Lucidum* in Inhibiting the Gene Expression of the *Leishmania Donovanii* Arginase Gene *In Vitro*

Hala Talib Abd Ali Al-Taei ^{*}, Yarub Modhar Al-Qazwini, Kiaser Abdalsajjad M. Hussain

Department of Biology, College of Education for pure Sciences, University of Kerbala, Karbala, Iraq

ARTICLE INFO

Article History:

Received 17 April 2026

Accepted 07 July 2026

Published 01 October 2026

Keywords:

Ganoderma lucidum

Leishmania donovani

Leishmania visceralis (VL)

Nanoparticles

ABSTRACT

Human visceral leishmaniasis is a severe parasitic disease caused by *Leishmania donovani* and is associated with high mortality worldwide. This study aimed to synthesize silver nanoparticles (AgNPs) using a green chemistry approach based on *Ganoderma lucidum* (Reishi mushroom) extract and evaluate their potential as a drug delivery system for Pentostam (Sb) against *L. donovani*. Silver nitrate solution (10 mM) was mixed with aqueous mushroom extract under controlled conditions (30–80 °C) to produce AgNPs, followed by loading with Pentostam (30 mg/mL). Successful synthesis and drug loading were confirmed using Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and atomic force microscopy (AFM). Promastigotes of *L. donovani* cultured in RPMI-1640 medium were treated with AgNPs loaded with Pentostam at different concentrations. The antileishmanial activity was assessed by one-step real-time PCR targeting the arginase (Arg) virulence gene. The AgNPs–Pentostam formulation (1 mL) exhibited the strongest inhibitory effect, reducing Arg gene expression to 0.24-fold (76% inhibition) relative to the untreated control. Free nanocomposite treatment resulted in a 0.36-fold change, whereas AgNPs–Pentostam (0.5 mL) produced a 0.49-fold change. These findings demonstrate that *G. lucidum*-mediated AgNPs loaded with Pentostam effectively suppress arginase gene expression in *L. donovani* and represent a promising nanotherapeutic strategy for visceral leishmaniasis.

How to cite this article

Al-Taei H., Al-Qazwini Y., M. Hussain K. Evaluating the Role of a Biosynthesized Nanocomposite Using the *Ganoderma Lucidum* in Inhibiting the Gene Expression of the *Leishmania Donovanii* Arginase Gene *In Vitro*. J Nanostruct, 2026; 16(4):4771-4780. DOI: 10.22052/JNS.2026.04.026

INTRODUCTION

Visceral leishmaniasis (VL) is the second deadliest parasitic disease after malaria. It is transmitted by vectors and caused by flagellated parasites of the genus *Leishmania*, specifically *L. donovani* and *L. infantum*, the two agents responsible for visceral leishmaniasis in the Old World, also known as *Leishmania donovani*-

complex parasites [1,2], The disease is widespread globally, particularly in developing countries, with approximately 300,000 new cases reported annually. It is prevalent in tropical and subtropical regions and is present in 98 countries across Europe, Africa, Asia, and the Americas [3].

The study [4] indicated that more than 90% of cases of visceral leishmaniasis occur in 13

^{*} Corresponding Author Email: hala.t@s.uokerbala.edu.iq



countries, and estimates suggest that between 0.9 and 1.7 million people are infected annually. The disease is spread by the bite of an infected sand fly of the genus *Phlebotomus* in the Old World and the genus *Lutzomyia* in the New World.

PCR tests targeted many conserved and variable regions of small DNA loops (KDNA) [5,6], genomic DNA, splice leader mini-exon (SLME) in parasitic cells [7], telomeric repeats [8], rRNA gene [9], to detect visceral parasite infection directly from infected human tissues.

Arginase is a metabolic and immunogenic virulence factor, and also a pathway for the synthesis of a compound Polyamines [10-12]. They are widespread aliphatic cations that play vital roles in a variety of essential cellular processes, including growth, differentiation, and macromolecule synthesis [13,14].

Sound pharmaceutical research and rigorous studies can lead to the discovery of more beneficial drugs. Available practical studies on a large number of plants and fungi, such as *Ganoderma lucidum*, indicate the potential for developing promising therapeutic biochemicals for many health problems. In herbal therapeutic approaches, a portion of an active extract or a mixture of such portions may be therapeutically superior, less toxic, and less expensive than pure, isolated compounds. However, crude plant and fungal preparations require modern standards for

safety and efficacy [15].

The study explained [16] The field of nanofabrication, also known as green chemistry, represents a revolution in the development of technologies for delivering treatments with high precision and efficiency. The most important characteristic of metallic nanoparticles is that they are submicron-sized, and therefore possess unique conductivity and loading properties due to their small size. These properties include a high surface area-to-volume ratio, a precise electronic structure, and the ability to interact with light through plasmon excitation. They can be synthesized using a variety of methods and modified with diverse chemical functional groups, allowing them to be combined with antibodies and drugs in biomedical applications. Furthermore, several microscopic examinations are conducted to confirm the success of their synthesis [17].

MATERIALS AND METHODS

Collection and culture of parasitic cells

Samples of *Leishmania donovani* parasite purified from infected patients' tissues by polymerase chain reaction (PCR) technology and cultured in NNN biphasic culture medium designated for their cultivation and nourishment were obtained from the laboratories of the Department of Life Sciences at Al-Mustansiriya University / Iraq. They were then purified and



Fig. 1. Stages of preparing the *Ganoderma lucidum* nanocomposite in the laboratory.

re-cultured in RPMI-1640 culture medium, and then incubated in a dedicated incubator at a temperature of 26°C, which is the appropriate temperature for the growth of the parasitic proflavostratus, until they were treated with *Ganoderma lucidum* nanoparticles.

Preparation of the *Ganoderma lucidum* nanocomposite

According to the study [18], under sterile laboratory conditions, 8 ml of silver nitrate solution (10 mM) was taken into a sterile glass flask and placed on a magnetic mixer for a quarter of an hour to ensure that the materials were well mixed together. After that, through a sterile burette, 4 ml of the aqueous fungal extract was distilled onto the silver nitrate solution at a temperature of 30–80 °C for 24 hours, observing the conditions of the process until the color of the compound changed to a dark, cloudy color. This indicates the complete reduction of silver ions and the formation of silver nanoparticles (AgNPs) [19], The nanocomposite is now ready, as shown in Fig. 1 and Fig. 2.

Loading Pentostam onto *Ganoderma lucidum* Nanocomposite

According to [20], the drug was loaded onto the aqueous extract and the nanocomposite as follows: Under sterile conditions, 10 ml of the nanocomposite was mixed with 0.5 ml of the drug, then the volume was increased to 20 ml (i.e., by adding 9.5 ml of deionized water) in a glass flask lined with silicone paper, The mixture was then placed on a thermocouple at a temperature not exceeding 50°C for 18 hours with magnetic stirring. After the specified time, the mixture was removed from the thermocouple. The mixture would have decreased to one-third (6.67%) due to stirring, Therefore, it was returned to the thermocouple after increasing the volume to 10 ml by adding deionized water, It was then left for 10 minutes to ensure homogeneity of the components. The process was then repeated, but this time with the addition of 1 ml of the drug to the same volume of nanocomposite mentioned above. Following this, the following steps were taken. Microscopic examinations AFM, SEM, FTIR

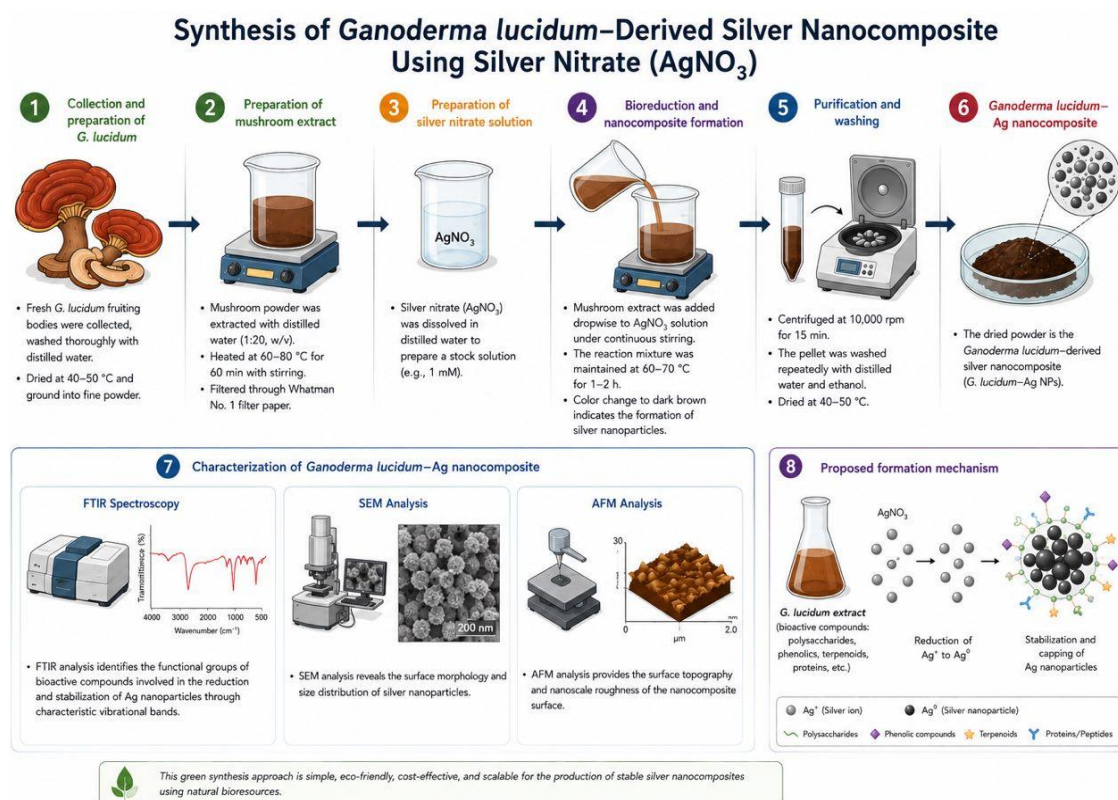


Fig. 2. Illustrates the steps for manufacturing the Reishi mushroom *Ganoderma lucidum* nanocomposite, as well as conducting microscopic examinations to show the properties of the compound and to ensure the success of its manufacturing process.

to confirm the success of the biosynthesis process of the nanocomposite.

Treatment of Parasitic Cells with Nanomedicine to Measure Gene Expression Levels

Parasitic proflagellate cells of *L.donovani* were treated with multiple volumetric additions of free nanocomposite and drug-loaded nanocomposite at 1 ml and 0.5 ml concentrations, respectively. Molecular assays, specifically polymerase chain reaction (PCR), were then performed. RNA was isolated and extracted from the parasitic cells using the TRIzol™ reagent protocol. 1.4 ml of parasite culture was isolated and precipitated by centrifugation for 2 minutes at 13,000 rpm. The culture medium was discarded, and 0.5 ml of TRIzol™ reagent was added to the precipitate. Residual culture medium was then removed from the extract by repeatedly aspirating and pushing it through with a pipette. 0.2 ml of chloroform was added to the precipitate in each tube, and the caps were tightly closed. The mixture was incubated for 2–3 minutes. The mixture was then centrifuged for 10 minutes at 12,000 rpm, with pH monitored at 4. The mixture was then separated into a lower cell phase, an interphase, and a colorless aqueous upper phase. The aqueous phase containing the RNA was transferred to a new tube. 0.5 ml of isopropanol was added to the aqueous phase, and the mixture was incubated for 10 minutes. It was then centrifuged for 10 minutes at 12,000 rpm. The total RNA precipitated, forming a white, gelatinous precipitate at the bottom of the tube. The supernatant was then removed. 0.5 ml of 70% ethanol was added to each tube, and the tubes

were briefly mixed using a vortex mixer. They were then centrifuged for 5 minutes at 10,000 rpm. Finally, the ethanol was removed, and the precipitate was left behind. To dry, rehydrate the precipitate in 50 microliters of nuclease-free water and then incubate in a water bath or heating block set to 55–60°C for 10–15 minutes.

Absolute Quantification Using the Standard Curve (SC)

The standard curve method is based on a series of dilutions of the template DNA copy number known in quantitative polymerase chain reaction (qPCR). The linear regression of the logarithm of the concentration (copies/μL) versus the threshold value (CT) gives the standard curve, which is used to calculate the template concentration (copies/μL) in the sample.

Statistical analysis

The statistical analysis program SAS 2012. Statistical Analysis System was used to detect the influence of the different factors in the study parameters, also the LSD Analysis of Variation-ANOVA test was used, the value of the least significant difference for the important comparison between the means used, In addition, SPSS and Excel 2010 were used.

RESULTS AND DISCUSSION

Diagnosis of therapeutic transaction Using microscopic examinations:

Fourier Transform Infrared (FTIR)

The results of the examination appear as curves showing the characteristics of those groups, and

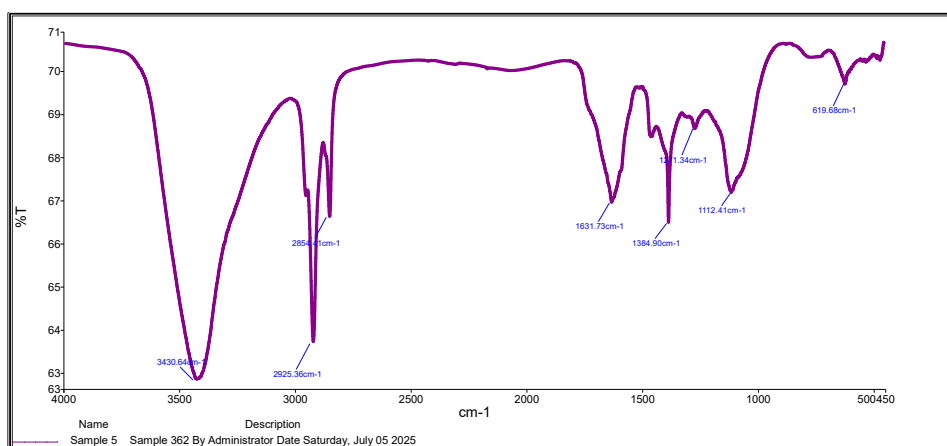


Fig. 3. Shows the FTIR infrared spectrum of the free fungal nanocomposite.

also identify the active structural classes in the compounds that contribute to the treatment process. To identify the active functional groups in the aqueous extract of Reishi mushroom, Fourier transform infrared spectroscopy was used.

Diagnosis of *Ganoderma lucidum* free-Nanocomposite

The FTIR analysis of the free fungal nanocomposite revealed varying wavelengths and frequencies, as shown in Fig. 3. The wavelengths range from 2854.41 nm to 619.68 nm. In its free state, the compound exhibits the highest and broadest absorption wavelength at 3430.64 nm. The analysis also showed a high peak at 2925.36 nm, followed by a series of high peaks. This characteristic suggests the compound's

therapeutic and drug delivery properties and indicates the success of its green biosynthesis. The activity of amino acids is observed at 3430.64 nm, while the wavelength at 1112.41 nm indicates C-H single-bonded alkanes. The wavelength curve 3430.64cm⁻¹ also shows C-H single-bonded aromatic rings and N-H amino compounds at frequency 3430.64cm⁻¹, while the wave at the same frequency 3430.64cm⁻¹ shows O-H single-bonded phenolic compounds and alcohols.

Diagnosis of the *Ganoderma lucidum* nanocomposite loaded with pentostam

FTIR spectroscopy results for the biosynthetic *Ganoderma lucidum* nanocomposite loaded with the drug Pentosam (SB) showed two waveforms with different frequencies. The first waveform

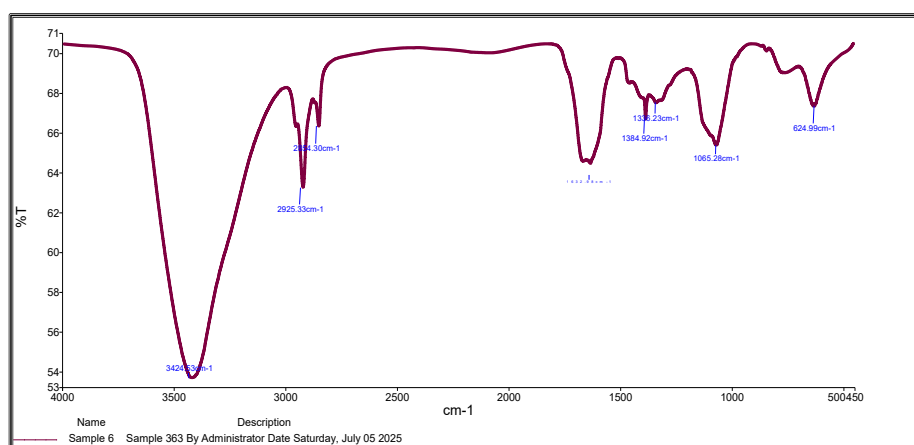


Fig. 4. Shows the FTIR infrared spectrum of the *Ganoderma lucidum* nanocomposite loaded with the drug Pentosam SB.

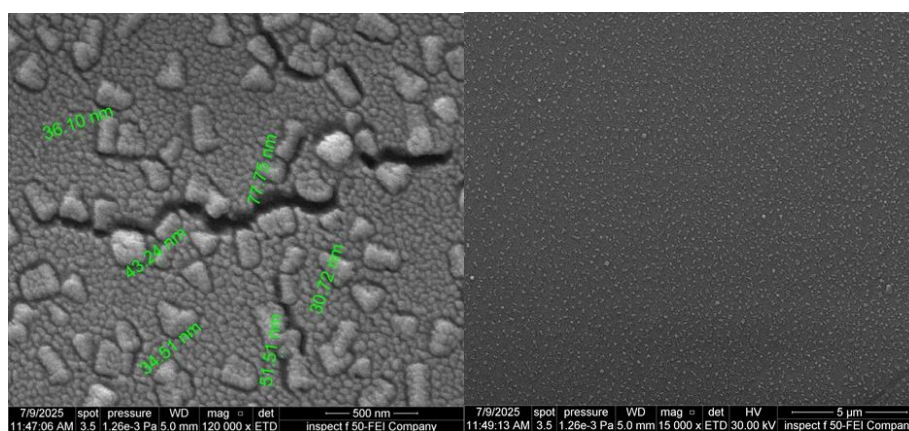


Fig. 5. Shows the results of scanning electron microscopy (SEM) examination of biosynthetic *Ganoderma lucidum* nanoparticles (GI+Nano).

was superimposed, starting at 2925.33 cm^{-1} and ending at 2354.30 cm^{-1} , while the second waveform included peaks between frequencies of 1632.98 cm^{-1} and 624.99 cm^{-1} . Fig. 4 shows that the resulting peaks were less intense and wider compared to the FTIR results for the free fungal nanocomposite shown in Fig. 3. This indicates the presence of drug molecules on the peaks of the nanocomposite, thus confirming the successful loading of Pentosam onto the biosynthetic compound. The green compound loaded with the drug also exhibited an absorption peak at a frequency of 3424.53 cm^{-1} . The wave with a frequency of (2925.33 cm^{-1}) indicated the highest and narrowest peak of the compound, while the wave with a frequency of (3424.53 cm^{-1}) revealed the N-H amino acid groups, and the same frequency (3424.53 cm^{-1}) indicated the C-H single-bonded aromatic cyclic groups, in addition to the phenolic compounds and O-H alcohols, while the C=C double-bonded alkene group was at the frequency of (1632.98 cm^{-1}).

Scanning Electron Microscopy (SEM)

Diagnosis of free nano-composite *Ganoderma lucidum*

The results of the microscopic examination showed good uniformity and dispersion of the particles on the surface of the nanocomposite, with the dominance of the spherical shape with a smooth shell, which is a distinctive feature in terms of the effectiveness of the Reishi mushroom nanocomposite, as the particle sizes range between ($77.75\text{ nm} - 30.72\text{ nm}$) nanometers,

with an average of 45.635 nm , which is within the nanoscale range ($60\text{--}22\text{ nm}$), at a magnification of $5000\text{ }\mu\text{m}$, 500 nm micrometers, as shown in Fig. 5.

Diagnosis of the aqueous extract of *Ganoderma lucidum* loaded with pentostam

The SEM results indicated homogeneity and harmonious distribution of the drug particles (SB) on the bio-synthesized *G. lucidum* nanocomposite particles, with a predominance of a near-spherical shape, but with smooth, flat surfaces. The particle dimensions ranged between ($234.8\text{ nm} - 314.1\text{ nm}$) with an average of (272.25 nm). These sizes fall within a larger range than nanoscale sizes ($60\text{--}22\text{ nm}$). Compared to the particle sizes in Fig. 6, this indicates the success of the drug loading process onto the green nanocomposite, at magnifications of 500 nm and $20000\text{ }\mu\text{m}$ micrometers, as shown in Fig. 6.

Atomic Force Microscope (AFM)

Diagnosis of free nano-composite *Ganoderma lucidum*

The results of the AFM (Automatic Microscopy) examination of the free nanocomposite showed that the surface roughness index of the extract particles was 6.509 nm , while its root mean square (RMS) was 8.076 nm , and the maximum peak height was 33.93 nm , while the maximum crater height was 28.44 nm . The results also showed that the degree of protrusion distribution was 0.02566 , with a maximum height of 62.37 nm , and the percentage of developed interfacial space was 14.01% . The surface illumination value was 2.700

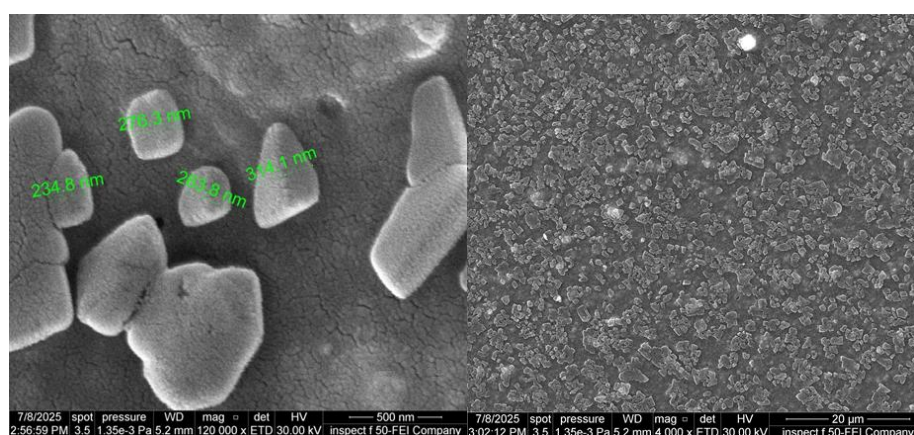


Fig. 6. Shows the results of scanning electron microscopy (SEM) examination of the nanoparticles of the *Ganoderma lucidum* compound loaded with the drug Pentosam (GI-Nano + SB) at magnification of 500 nm and $20000\text{ }\mu\text{m}$.

nm, the texture direction was 176.7° , and the surface slope was 0.5822. The current results also indicated that the average size of the biosynthetic composite under study was 31.94 nm, as shown in Fig. 7.

Diagnosis of the aqueous extract of *Ganoderma lucidum* loaded with pentostam

Three-dimensional aperture microscopy (AFM) of the fungus nanoparticles loaded with Pentosam SB revealed that the surface roughness of the aforementioned therapeutic material particles

was 8.150 nm, with a root mean square (RMS) of 10.43 nm. The maximum apex height was 30.69 nm, and the maximum crater height was 32.34 nm. The results also showed that the degree of protrusion distribution was -0.3405, with a maximum protrusion height of 63.03 nm. The improved interfacial area was 6.099%, and the surface illumination was 3.219 nm. The texture orientation was 0.009062° , with a surface slope of 0.3680. The microscopic results indicated that the average size of the Reishi fungus nanoparticles loaded with the drug The SB used in the current

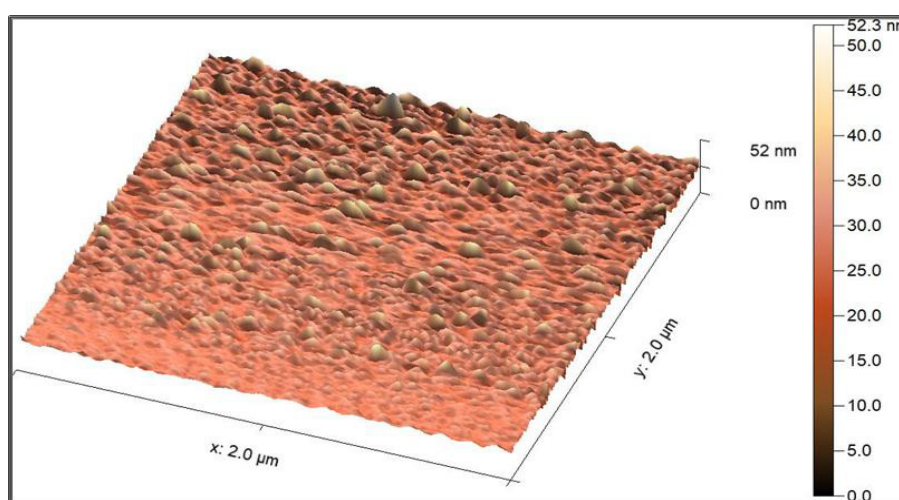


Fig. 7. Shows a three-dimensional image using AFM technology of bio-synthesized *Ganoderma lucidum* nanoparticles.

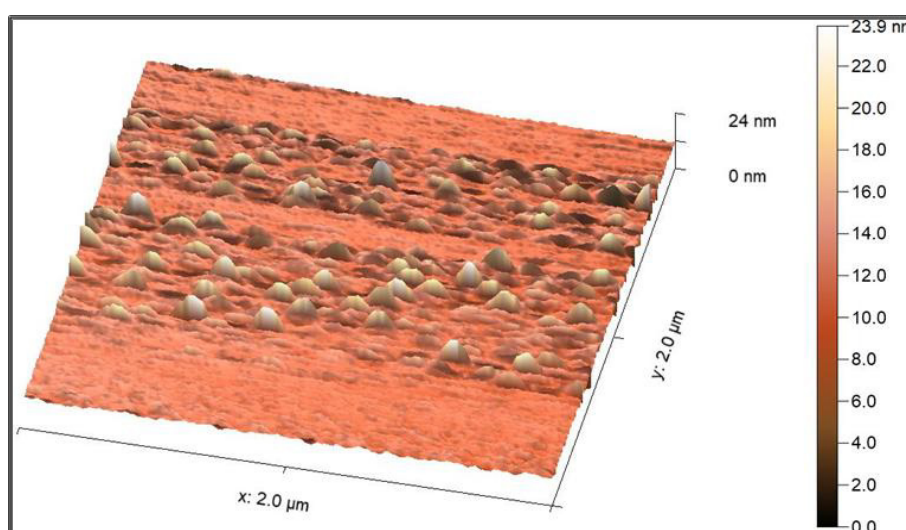


Fig. 8. Shows a three-dimensional image using AFM technology of biosynthetic *Ganoderma lucidum* nanoparticles loaded with the drug Pentosam SB.

study was 45.12 nanometers, as shown in Fig. 8.

Effect of therapeutic treatments on arginase (Arg) enzyme in the promastigotes cells of Leishmania donovani parasite

Primary parasitic cells were treated with various volumes of therapeutic treatments, namely: free nanocomposite of *G.lucidum*, fungal nanocomposite + SB drug 1ml, fungal nanocomposite + SB drug 0.5ml. The study adopted the following three volumetric additions: (100 μ l - 75 μ l - 25 μ l) with three replicates for each volumetric addition of the drug spectrums used.

The results of the examination under the influence of treatment with multiple drug spectra showed varying degrees of activity in the level of gene expression regulation of the Arg virulence gene of the parasite at a significance level of

$P \leq 0.05$ compared to the reference normal value for the N-control group, which was the basis of comparison, consisting of a cell suspension containing parasitic promastigotes of the visceral *Leishmania donovani* parasite that was not treated with the drug spectra under study. The difference between the multiple volumetric additions of the treatments (copies/ μ L) on the level of Arg gene synthesis activity within the primary cells was represented by an average fold change of 1.00, as shown in Table 1, Sample B(Nano + SB 1ml) with its three volumetric additions achieved the strongest gene inhibition rate of 0.24 V-fold change, meaning that treatment C reduced the level of gene expression downregulation of the Arg virulence gene by an approximate percentage, It reached (76%) as shown in Fig. 9, followed by sample A (Free Nano) with a strong decrease

CT

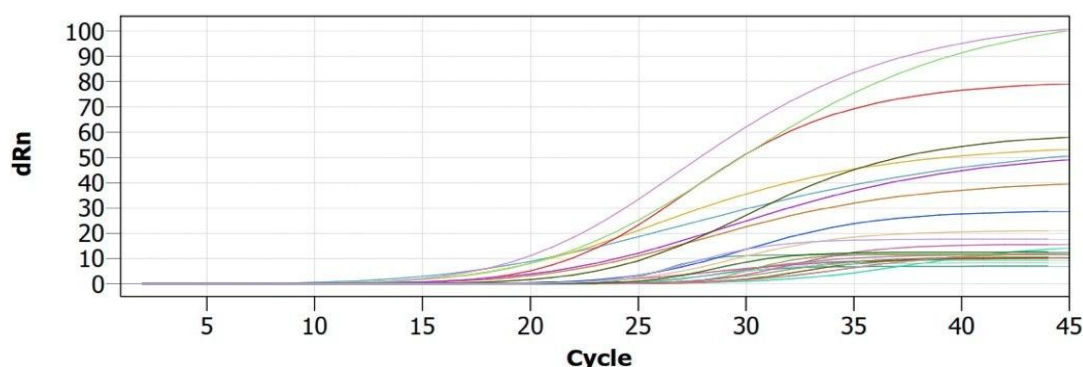


Fig. 9. Shows the amplification plot of the difference in gene expression of the Arg gene, where the first three lines represent the control group elements, while the other lines represent the treatment samples. Horizontal axis (Cycle): Number of reaction cycles. Vertical axis (dRn): Fluorescence intensity (reflects the amount of DNA replicated). Each colored curve represents a different treatment.

Table 1. Shows the values of change in gene expression regulation calculated after treatment with the multiple drug spectra under study.

	Treatment	Dose(μ l)	Copies/ μ l	v- Fold Change	Mean $\pm$ SD*
A	(Nano)	100	0.310	0.36	0.36 $\pm$ 0.05c
		75	0.374		
		25	0.395		
B	(N+Sb 1ml)	100	0.148	0.24	0.26 $\pm$ 0.13d
		75	0.235		
		25	0.403		
C	(N+Sb 0.5ml)	100	0.451	0.49	0.49 $\pm$ 0.05bc
		75	0.476		
		25	0.547		
	Control	-----	1.414	1.00	1.13 $\pm$ 0.56 a*
		-----	1.526		
		-----	1.463		

$P \leq 0.05$). (* Represents a probability level

in gene expression with a percentage of (V-fold change = 0.36), while sample C (Nano + SB 0.5ml) showed a change in the expression of the arginase gene with a percentage of (V-fold change = 0.49).

In the quantitative analysis of genetic material, a graph was used to analyze the results of quantitative qPCR to measure the amount of DNA after amplification in the multiple samples used in the study, as shown in Fig. 9. The signal starts low (baseline) and then gradually increases exponentially depending on the amount of amplified DNA, eventually reaching saturation.

It is worth noting that the CT (Cycle Threshold) value is the point at which the fluorescence signal begins to rise significantly. This value is measured by the qPCR instrument and is used to estimate the amount of amplified genetic material in the sample. Furthermore, the CT value is used to calculate the level of fold change in gene expression and to determine whether expression has increased (upregulation) or decreased (downregulation).

It is worth noting that the lower the percentage of gene activity, the stronger the therapeutic treatment is in influencing the regulation of gene expression of the Arg virulence gene of the visceral parasite under the influence of treatment with drug spectrums.

Based on the results in Table 1 above, we find that all treatment treatments affected, to varying degrees, the normal expression level of the Arg gene within the parasitic proflavocardia cells, We also observe that the fungal nanoparticle loaded with the drug at a concentration of 1 ml, as well as the free nanoparticle with all their volumetric additions, showed a direct and highly potent effect in inhibiting the gene expression level of the Arg gene within the parasitic cells, This may be attributed to the high efficacy of nanoparticles in penetrating the defenses of the parasitic cells and their ability to damage their genetic material, a finding consistent with the study [21], The study also demonstrated varying degrees of change in the gene expression of virulence genes that resist the host's immune defenses, in addition to the synergistic effect of the pentosam drug Sb, which is characterized by its high toxicity to the visceral parasite *L. donovani* [22].

CONCLUSION

This study demonstrates the potential of a biosynthesized *Ganoderma lucidum*-based

silver nanocomposite, alone and in combination with Pentostam, as a promising anti-leishmanial strategy against *Leishmania donovani* promastigotes. Physicochemical characterization by FTIR, SEM, and AFM confirmed the successful green synthesis of the fungal nanocomposite and the effective loading of Pentostam onto its surface. The observed spectral shifts, altered peak intensities, and changes in particle morphology and size after drug loading collectively indicate stable interaction between the nanocomposite and the drug, supporting its suitability as a delivery platform. Biologically, all tested treatments reduced the expression of the arginase (Arg) virulence gene relative to the untreated control, indicating a measurable inhibitory effect on a key metabolic pathway associated with parasite survival, polyamine biosynthesis, and pathogenicity. Among the tested formulations, the *Ganoderma lucidum* nanocomposite loaded with 1 ml Pentostam exhibited the strongest suppression of Arg expression, achieving a fold change of 0.24, equivalent to approximately 76% downregulation. The free nanocomposite also showed marked inhibitory activity, suggesting that the fungal nanomaterial itself possesses intrinsic antiparasitic properties, while drug loading further enhances efficacy through a likely synergistic effect. Taken together, these findings support the concept that green-synthesized fungal nanocomposites can serve both as bioactive therapeutic agents and as efficient nanocarriers for conventional drugs. This dual functionality may improve treatment performance against visceral leishmaniasis while potentially reducing toxicity and enhancing targeted action. Further studies are required to validate these results in vivo, clarify the molecular mechanisms involved, and assess safety, dosage optimization, and clinical applicability.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

REFERENCES

1. Al-Tae MSAH, Al-Qazwini YM, Mikhlef HZ. Effect of Volatile Oil Extract of Two Plants (*Lavandula angustifolia*, *Cupressus sempervirens*) on the Vitality of Visceral Leishmaniasis. *Lecture Notes in Networks and Systems: Springer Nature Singapore*; 2025. p. 559-568. http://dx.doi.org/10.1007/978-981-96-3244-2_44
2. Al-Taei HTAA, Al-Qazwini YM, Zwaier H. Evaluation of inhibitory

- effect of Saliva officinalis extracts and volatile oils on the life of *Leishmania donovani* parasite. *International journal of health sciences*. 2022;2015-2024.
3. Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. Leishmaniasis Worldwide and Global Estimates of Its Incidence. *PLoS One*. 2012;7(5):e35671.
 4. Steverding D. The history of leishmaniasis. *Parasites and Vectors*. 2017;10(1).
 5. Maurya V, Guhnani HC, Sarma PU, Madan T, Shah A. Sensitization to *Aspergillus* Antigens and Occurrence of Allergic Bronchopulmonary Aspergillosis in Patients With Asthma. *Chest*. 2005;127(4):1252-1259.
 6. Salotra P, Sreenivas G, Pogue GP, Lee N, Nakhasi HL, Ramesh V, et al. Development of a Species-Specific PCR Assay for Detection of *Leishmania donovani* in Clinical Samples from Patients with Kala-Azar and Post-Kala-Azar Dermal Leishmaniasis. *J Clin Microbiol*. 2001;39(3):849-854.
 7. Ramos VA, Cegarra M, Cristallini E. Cenozoic tectonics of the High Andes of west-central Argentina (30–36°S latitude). *Tectonophysics*. 1996;259(1-3):185-200.
 8. Chiurillo MA, Sachdeva M, Dole VS, Yepes Y, Miliani E, Vazquez L, et al. Detection of *Leishmania* causing visceral leishmaniasis in the Old and New Worlds by a polymerase chain reaction assay based on telomeric sequences. *The American journal of tropical medicine and hygiene*. 2001;65(5):573-582.
 9. Guillaume J.J.M vE, Schoone GJ, Kroon NCM, Ebeling SB. Sequence analysis of small subunit ribosomal RNA genes and its use for detection and identification of *Leishmania* parasites. *Molecular and Biochemical Parasitology*. 1992;51(1):133-142.
 10. Bacchi CJ, Nathan HC, Clarkson AB, Bienen EJ, Bitonti AJ, McCann PP, et al. Effects of the Ornithine Decarboxylase Inhibitors Di- α -Difluoromethylornithine and α -Monofluoromethyldehydroornithine Methyl Ester Alone and in Combination with Suramin against *Trypanosoma brucei brucei* Central Nervous System Models. *The American Journal of Tropical Medicine and Hygiene*. 1987;36(1):46-52.
 11. Jiang Y, Roberts SC, Jardim A, Carter NS, Shih S, Ariyanayagam M, et al. Ornithine Decarboxylase Gene Deletion Mutants of *Leishmania donovani*. *J Biol Chem*. 1999;274(6):3781-3788.
 12. Fairlamb AH. Chemotherapy of human African trypanosomiasis: current and future prospects. *Trends Parasitol*. 2003;19(11):488-494.
 13. Bachrach CA. Culture and Demography: From Reluctant Bedfellows to Committed Partners. *Demography*. 2013;51(1):3-25.
 14. Tabor CW, Tabor H. POLYAMINES. *Annual Review of Biochemistry*. 1984;53(1):749-790.
 15. *Ganoderma lucidum*: A Potent Medicinal Mushroom with Numerous Health Benefits. *Pharmaceutica Analytica Acta*. 2013;04(10).
 16. Eagderi S, Mouludi-Saleh A, Esmaeli HR, Sayyadzadeh G, Nasri M. Freshwater lamprey and fishes of Iran; a revised and updated annotated checklist-2022. *Turkish Journal of Zoology*. 2022;46(6):500-522.
 17. Mody V, Siwale R, Singh A, Mody H. Introduction to metallic nanoparticles. *Journal of Pharmacy And Bioallied Sciences*. 2010;2(4):282.
 18. Youssef MA, Hamza NM, Hussain KAM. The effect of aqueous extract of *Cinnamomum zeylanicum* on some biochemical parameters in male white rats in which rheumatoid arthritis is induced. *AIP Conference Proceedings: AIP Publishing*; 2023. p. 020031.
 19. Al-Karawi RH, Al-Safy AH, Hussain KAM. Evaluation of the biological efficacy of green moringa nanocomposite in pregnant female rats treated with ibuprofen. *AIP Conference Proceedings: AIP Publishing*; 2026. p. 030051.
 20. Pereira MB, Sydor BG, Memare KG, Verzignassi Silveira TG, Alessi Aristides SM, Dalmarco EM, et al. In Vivo Efficacy of Meglumine Antimoniate-Loaded Nanoparticles for Cutaneous Leishmaniasis: A Systematic Review. *Nanomedicine*. 2021;16(17):1505-1518.
 21. Shnawa BH, Mhammedsharif RM, Jalil PJ, Hamadamin SI, Ahmad SF, Abdulrahman KM, et al. Antimicrobial activity of plant-extract-mediated synthesis of Silver-Zinc Oxide nanocomposites and their acaricidal efficacy on *Hyalomma marginatum* ticks. *Biocatalysis and Agricultural Biotechnology*. 2023;51:102765.
 22. Mohtasebi S, Mohebbi M, Elikae S, Akhoundi B, Foroushani AR, Teimouri A, et al. In vitro and in vivo anti-parasitic activity of biogenic antimony sulfide nanoparticles on *Leishmania major* (MRHO/IR/75/ER). *Parasitol Res*. 2019;118(9):2669-2678.