Iranian J Biotech. 2020 January;18(1): e2196 DOI: 10.30498/IJB.2020.119481.2196

Research Article

Greener Synthesis of Rod Shaped Zinc Oxide NPs Using *Lilium ledebourii* Tuber and Evaluation of Their Leishmanicidal Activity

Mehrdad Khatami[1,2], Sanaz Khatami[3], Farideh Mosazade[1], Mahammadali Raisi[4], Mojtaba Haghighat[5], Mohamad Sabaghan[*5], Sajad Yaghoubi[6], Mina Sarani[7], Mehdi Bamorovat[8], Leila Malekian[4], Afsoon Naroi[9], Rajender S. Varma[10].

[1] Noncommunicable Diseases Research Center, Bam University of Medical Sciences, Bam, Iran
[2] Cell Therapy and Regenerative Medicine Comprehensive Center, Kerman University of Medical Sciences, Kerman, Iran
[3] Department of Medical Biotechnology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[4] Clinical Research Center, Pastor Educational Hospital, Bam University of Medical Sciences, Bam, Iran
[5] Behbahan Faculty of Medical Sciences, Behbahan, Iran
[6] Department of Clinical Microbiology, Iranshahr University of Medical Sciences, Iranshahr, Iran
[7] Zabol Medicinal Plants Research Center, Zabol University of Medical Sciences, Zabol, Iran
[8] Leishmaniasis Research Center, Kerman University of Medical Sciences, Kerman, Iran
[9] Student Research Committee, School of Public Health, Bam University of Medical Sciences, Bam, Iran
[10] Regional Centre of Advanced Technologies and Materials Faculty of Science, Palacky University in Olomouc, Olomouc, Czech Republic

[*] *Corresponding author:* Dr. Mohamad Sabaghan, Behbahan Faculty of Medical Sciences, Behbahan, Iran. E-mail: M.sabaghan@behums.ac.ir

Background: NPs (NPs) with unique chemical and physical properties can be used for therapeutic purposes because of their strong antimicrobial activates. NPs have been used as an antimicrobial agents to inhibit microbial growth.
Objectives: In view of the strong antimicrobial activity of NPs, the biogenic synthesis and leishmanicidal activity of rod-shaped zinc oxide (R-ZnO) NPs was explored using *Lilium ledebourii* tuber extract.
Materials and Methods: The ensuing NPs are characterized by UV-visible spectroscopy, X-ray diffraction and transmission electron microscopy and their leishmanicidal activity evaluated against the *Leishmania major* (*L. major*) by MTT assay.
Results: The R-ZnO NPs displayed excellent leishmanicidal activity against the *L. major* as they significantly inhibited the amastigotes. The IC_{50} values of R-ZnO NPs being ~ 0.001 $mg.mL^{-1}$. R-ZnO NPs can inhibit *L. major* growth in a dose-dependent manner under in vitro conditions.
Conclusion: A simple, low-cost feasible and eco-friendly procedure was developed for biosynthesis of R-ZnO NPs using natural bioresource that can inhibit human parasite cells growth in a dose-dependent manner under *in vitro* conditions.
Keywords: Biosynthesis, Leishmanicidal, NPs, Rod-shaped, Zinc Oxide.

1. Background

Leishmaniasis is a disease caused by *Leishmania* species with the incidence rate of about two million cases annually (1). Currently, the increase in international travel and environmental amendments caused by war in some regions has created favorable conditions for the propagation of the parasite vectors; thus, there has been a significant increase in the incidence of leishmaniasis (2). Poverty contributes to the risk of leishmaniasis and so does the open sewerage and lack of waste management which can increase sandfly breeding sites of Leishmania vectors, as well as their access to human bodies. About 70 animal species have been found to act as natural reservoir hosts for these parasites (3). The first choice for the treatment of leishmaniasis is pentavalent antimoniate (Pa). But the Pa—because of its side effects, the emergence of resistance, and parenteral application is no longer a sufficient treatment. Therefore, it is essential to find new drugs with different mechanisms ofand greater potency (4).

Nanoparticles (NPs) can be used for therapeutic applications because of their strong antimicrobial activities (5-7). Such as in drug delivery, microbiology, biotechnology, and biochemistry (8-15). The use of metal NPs such as copper, platinum , titanium , gold, selenium, silver, zinc oxide, and palladium NPs against many bacteria, viruses, and fungal pathogens has been reported (16-19), but the use of NPs against protozoan parasites is rather limited. Among different types of NPs, the zinc oxide (ZnO) NPs have attracted scientific attention because of their safety and high antimicrobial activity (20, 21); they are recognized to be safe by the U.S. Food and Drug Administration (FDA). The traditional NPs synthesized by chemical methods leads to the adsorption of toxic chemical compounds onto the surface of synthesized NPs which may have adverse effects in medicine (22, 23). Green synthesis methods using bioresources such as plants, fungi, or bacteria for the synthesis of biogenic NPs represent alternatives to conventional chemical synthesis methods (24-29).

The present study aims to evaluate the leishmanicidal efficiency of rod-shaped ZnO (R-ZnO) NPs on *Leishmania major* cultures.

2. Objectives

The main aim of this study was to evaluate green synthesis of parasitical zinc oxide NPs using *Lilium ledebourii* tuber. A greener synthesis of rod shape zinc oxide (R-ZnO) NPs were studied using *L. ledebourii* tuber as a novel bioresource and their leishmanicidal activity has been studied against *L. major*.

3. Materials and Methods

3.1. Materials

All the reagents and chemicals used in the experiments were purchased from Merck, Germany.

3.2. Synthesis of Rod Shaped ZnO NPs

The *L. ledebourii* tuber were washed thoroughly with sterile distilled water and dried, then 10 g of Lilium tuber was ground into a powder. The powder was added to 200 mL of deionized double-distilled water, heated at 80 °C, and then filtered. To conduct green synthesis of R-ZnO NPs, 20 mL of the obtained extract was added to the 100 mL of zinc acetate dehydrate solution and stirred at ~80 °C for 1 hr. The reaction mixture (extract + zinc acetate dehydrate) was incubated at 80 °C for 2 hr and calcined at 300 °C for 1 hr.

3.3. Characterization of ZnO NPs

The synthesized ZnO NPs were studied using a UV-visible spectrophotometer (Analytik Jena; Germany). XRD analysis was performed to determine the formation of ZnO crystals. The resulting powder was analyzed using an X-ray diffractometer (PANalytical X'Pert PRO; The Netherlands) at 2θ. The shape, size and size distribution of nanoparticle were investigated by TEM (30).

3.4. Leishmanicidal Assay

The *L. major* MRHO/IR/75/ER standard strain was cultured in RPMI1640 at 25 °C supplemented with 15% heat-inactivated FBS, streptomycin (200 $mg.mL^{-1}$), and penicillin (200 $IUmg.mL^{-1}$). The stationary growth phase of promastigotes was added to the macrophages to generate a parasite/macrophage ratio of 10:1. It was then incubated at 37 °C in 5% CO_2 for 24 hr (31). The macrophages containing antiamastigote were treated with various concentrations of 0–500 $mg.mL^{-1}$ R-ZnO NPs (32).

3.5. Statistical Analysis

The differences between the groups were determined using one-way analysis of variance (ANOVA) to analyse the obtained results. A *p*-value < 0.05 was considered significant.

4. Results

4.1. Biosynthesis and Characterization of ZnO NPs

The UV-Vis spectrum at 350–370 nm wavelengths (**Fig. 1**) shows the synthesis of R-ZnO NPs to be consistent with previous findings (33**).**

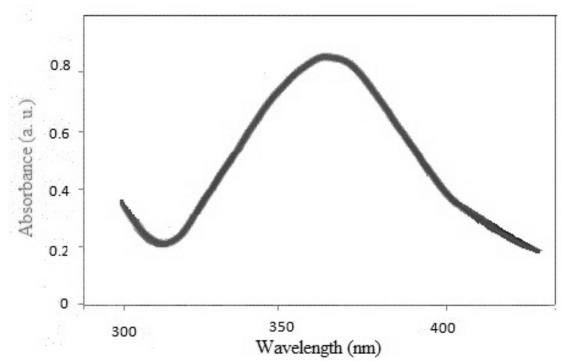

Fig. 1. UV-visible spectroscopy of biosynthesized ZnO nanoaprticles

The XRD pattern shows dispersion peaks at 31, 35, 37, 48, 57, 62, 66, 68, and 69, thus confirming the presence of ZnO NPs in the sample (**Fig. 2**). which corroborated previous with previous findings (33, 34).

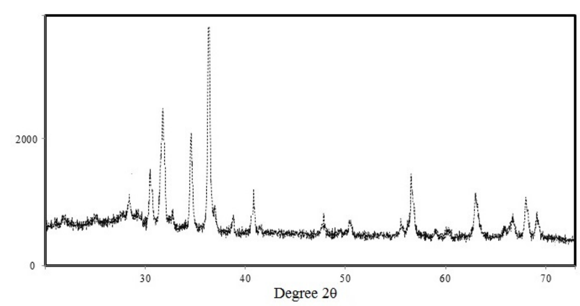

Fig. 2. XRD pattern of biosynthesized R-ZnO nanoaprticles.

TEM images confirm that biogenic ZnO NPs have rod-shaped morphology; however, spherical NPs were observed in the TEM images. R-ZnO NPs are below 100 nm in size (**Fig. 3**).

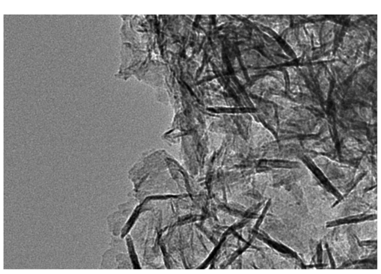

Fig. 3. TEM image of biosynthesized R-ZnO NPs nanoaprticles.

4.2. Antiamastigote Assay

The R-ZnO NPs were found to inhibit the multiplication rate of amastigotes in a dose-dependent manner (**Fig. 4**). The IC_{50} values were about 10 $mg.mL^{-1}$ for both R-ZnO NPs and Meglumine antimoniate (glucantime) as positive control. The concentration of 0.5 $mg.mL^{-1}$ of biogenic R-ZnO NPs revealed a higher toxicity effect on *L. major* (amastigotes). The results disclose that by increasing the concentration of R-ZnO NPs, the viability of the tested parasites significantly decreases.

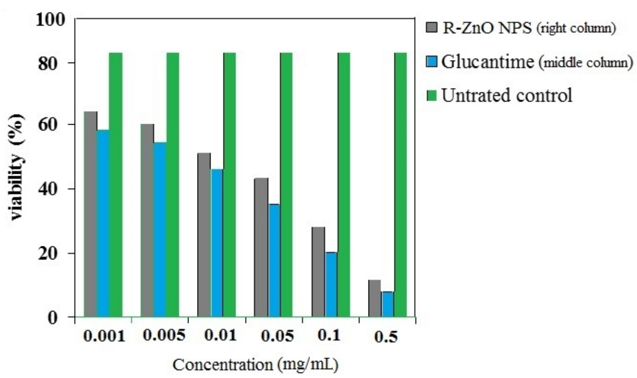

Fig. 4. The effect of R-ZnO NPs and Glucantime on viability of *Leishmania major* (amastigotes).

5. Discussion

In this study, for the first time, the rod shaped ZnO NPs were biosynthesized as a safe therapeutic nanomaterial. A novel protocol for the greener production of the rod shaped ZnO NPs is presented in this study. The traditional NPs synthesized by chemical methods leads to the adsorption of toxic chemical compounds onto the surface of synthesized NPs which may have adverse effects in medicine. The NPs that are produced by plant extract have lower environmental risk due to lack of harsh chemicals often in their synthesis process. Therefore, they can be applied in medical programs such as drug delivery. The salient advantages of producing plant NPs via greener methods such as using bacterial or fungal extracts is the safety and high availability of medicinal plants that are more reliable and healthier than bacterial or fungal extracts mediated for the production of NPs. Green synthesis methods using bioresources such as plants, fungi, or bacteria for the synthesis of biogenic NPs represent alternatives to conventional chemical synthesis methods

The use of factors present in the plant and fungus residue not only are responsible in the synthesis of NPs, but by stacking around the NPs, they cause stability and prevent their agglomoration. Plants, in addition to being non-toxic, have different types of metabolites, which can be effective in the synthesis of NPs which can include terpenoids, flavonoids, carbonyls, amides, and carboxylic acids, which directly contribute to the formation of NPs.

The result of a study conducted against *L. major* showed that rod shaped ZnO NPs have good leishmanicidal activity against *L. major* and this outcome could help in the development of formulations as efficient means to synthesize R-ZnO NPs from natural products in our fight against the resistant microorganisms (35, 36).

In our study, the rod shaped ZnO NPs displayed strong leishmanicidal efficiency (IC_{50} about 0.012 $mg.mL^{-1}$). There are no reports on the leishmanicidal activity of biogenic rod shaped ZnO NPs nor on the cytotoxic effect of chemically synthesized Rod shaped ZnO NPs on living cells. The study by Delavari *et al.* 2014 (37) have reported leishmanicidal activity of chemicaly synthesized spherial shaped ZnO NPs on L. major, the IC_{50} ZnO NPs being 0.0378 $mg.mL^{-1}$ on promastigotes of L. major. Also Sumaira *et al*. (38) have reported leishmanicidal activity od spherical greener sythsized ZnO NPs, the IC_{50} ZnO NPs being 0.25 $mg.mL^{-1}$ against *L. tropica.* But in our present study, the biogenic greener synthesized NPs rod shaped ZnO NPs displayed much stronger leishmanicidal activity (IC_{50} about 0.012 $mg.mL^{-1}$); they are more effective in leishmanicidal activity compared to chemically assembled spherical shape ZnO NPs or greener spherial shaped ZnO nanoarticles. Additionally, biogenic R-ZnO NPs can also be used *in vivo*.

6. Conclusion

These results show that it is possible to prepare a safe and ecofriendly synthsized NPs with leishmancidal potential. The greener synthesized rod shaped ZnO NPs, displayed stronger leishmanicidal activity (IC50 about 0.012 $mg.mL^{-1}$); compared to chemically assembled spherical shape ZnO NPs and greener spherical shaped ZnO NPs (37).

Acknowledgements

This project was reviewed and approved and financed by the Behbahan and Bam Universities of Medical Sciences.

Competing interests

The authors confirm that this article content has no competing interests.

Authors' Contribution

All authors have participated in the manuscript preparation.

Financial Disclosure

There is no conflict of interest

References

1. Khatami M, Alijani H, Sharifi I, Sharifi F, Pourseyedi S, Kharazi S, *et al*. Leishmanicidal Activity of Biogenic Fe_3O_4 NPs. *Sci Pharm*. 2017;**85**(4):36. doi: 10.3390/scipharm85040036.
2. Aflatoonian MR, Sharifi I, Aflatoonian B, Bamorovat M, Heshmatkhah A, Babaei Z, *et al*. Associated-risk determinants for anthroponotic cutaneous leishmaniasis treated with meglumine antimoniate: A cohort study in Iran. *PLoS Negl Trop Dis.* **13**(6): e0007423. doi: 10.1371/journal.pntd.0007423.
3. Bamorovat M, Sharifi I, Aflatoonian MR, Sadeghi B, Shafiian A, Oliaee RT, *et a*l. Host's immune response in unresponsive and responsive patients with anthroponotic cutaneous leishmaniasis treated by meglumine antimoniate: A case-control study of Th1 and Th2 pathways. *Int immunopharmacology*. 2019;**69**:321-327. doi: 10.1016/j.intimp.2019.02.008.
4. Daneshvar H, Tavakoli Kareshk A, Sharifi I, Keyhani A, Tavakoli Oliaee R, Asadi A. Host-parasite Responses Outcome Regulate the Expression of Antimicrobial Peptide Genes in the Skin of BALB/c and C57BL/6 Murine Strains Following Leishmania major MRHO/IR/75/ER Infection. *Iran J Parasitol.* 2018;**13**(4):515-523. doi: 10.1016/j.ijbiomac.2017.08.149.
5. Mahmoudvand H, Fasihi Harandi M, Shakibaie M, Aflatoonian MR, ZiaAli N, Makki MS, *et al*. Scolicidal effects of biogenic selenium NPs against protoscolices of hydatid cysts. *Int J Surg*. 2014;**12**(5):399-403. doi: 10.1016/j.ijsu.2014.03.017.
6. Jahani S, Khorasani-Motlagh M, Noroozifar M. DNA interaction of europium(III) complex containing 2,2′-bipyridine and its antimicrobial activity. *J Biomol Struct Dynamics.* 2016;**34**(3):612-624. doi: 10.1080/07391102.2015.1048481.
7. Karthik K, Vijayalakshmi S, Phuruangrat A, Revathi V, Verma U. Multifunctional Applications of Microwave-Assisted Biogenic TiO_2 NPs. *J Clu Sci*. 2019;**30**:1-8. doi: 10.1007/s10876-019-01556-1
8. Niroomand S, Khorasani-Motlagh M, Noroozifar M, Jahani S, Moodi A. Photochemical and DFT studies on DNA-binding ability and antibacterial activity of lanthanum(III)-phenanthroline complex. *J Mol Struct.* 2017;**1130**:940-950. doi: 10.1016/j.molstruc.2016.10.076.
9. Rahi A, Sattarahmady N, Heli H. Zepto-molar electrochemical detection of Brucella genome based on gold nanoribbons covered by gold nanoblooms. *Sci Rep.* 2015;**5**:18060. doi:10.1038/srep18060.
10. Hossein H, Masoud N. Applications of Nanoflowers in Biomedicine. *Recent Pat Nanotechnol* 2018;**12**(1):22-33. doi: 10.2174/1872210511666170911153428.
11. Khatami M, Iravani S, Varma R.S, Mosazade F, Darroudi M, Borhani F. Cockroach wings-promoted safe and greener synthesis of silver NPs and their insecticidal activity. *Bioprocess Biosyst Eng.* 2019;**42**. doi: https://doi.org/10.1007/s00449-019-02193-8.
12. Akhtartavan S, Karimi M, Karimian K, Azarpira N, Khatami M, Heli H. Evaluation of a self-nanoemulsifying docetaxel delivery system. *Biomed Pharmaco*. 2019;**109**(2019):2427-2433. doi:10.1016/j.biopha.2018.11.110.
13. Rajaei M, Foroughi MM, Jahani S, Shahidi Zandi M, Hassani Nadiki H. Sensitive detection of morphine in the presence of dopamine with La3+ doped fern-like CuO nanoleaves/MWCNTs modified carbon paste electrode. *J Mol Liq*. 2019;**284**:462-472. doi: 10.1016/j.molliq.2019.03.135.
14. Torkzadeh-Mahani R, Foroughi MM, Jahani S, Kazemipour M, Hassani Nadiki H. The effect of ultrasonic irradiation on the morphology of NiO/Co_3O_4 nanocomposite and its application to the simultaneous electrochemical determination of droxidopa and carbidopa. *Ultrason Sonochem*. 2019;**56**:183-192. doi: 10.1016/j.ultsonch.2019.04.002.
15. Mohammadinejad R, Moosavi MA, Tavakol S, Vardar DÖ, Hosseini A, Rahmati M, et al. Necrotic, apoptotic and autophagic cell fates triggered by NPs. *Autophagy*. 2019;**15**(1):4-33. doi: 10.1016/j.ijbiomac.2017.08.149.
16. Darroudi M, Sarani M, Kazemi Oskuee R, Khorsand Zak A, Amiri MS. Nanoceria: Gum mediated synthesis and in vitro viability assay. *Ceram Int*. 2014;**40**(2):2863-2868. doi: 10.1016/j.ceramint.2013.10.026.
17. Sattarahmady N, Rezaie-Yazdi M, Tondro GH, Akbari N. Bactericidal laser ablation of carbon dots: An in vitro study on wild-type and antibiotic-resistant *Staphylococcus aureus*. *J Photochem Photobiol B.* 2017;**166**(Supplement C):323-332. doi: 10.1016/j.jphotobiol.2016.12.006.
18. Karthik K, Dhanuskodi S, Gobinath C, Prabukumar S, Sivaramakrishnan S. Fabrication of MgO nanostructures and its efficient photocatalytic, antibacterial and anticancer performance. *J Photochem Photobiol B.* 2019;**190**:8-20. doi: 10.1016/j.jphotobiol.2018.11.001.
19. Khatami M, Alijani H, Sharifi I. Biosynthesis of bimetallic and core shell NPs: their biomedical applications: A review. *IET Nanobio*. 2018;**12**(7): 879-887. doi: 10.1049/iet-nbt.2017.0308.
20. Dağlıoğlu Y, Yılmaz Öztürk B. Effect of concentration and exposure time of $ZnO-TiO_2$ nanocomposite on photosynthetic pigment contents, ROS production ability, and bioaccumulation of freshwater algae (Desmodesmus multivariabilis). *Caryologia*. 2018;**71**(1):13-23. doi: 10.1080/00087114.2017.1400262
21. Khatami M, Varma R.S, Heydari M, Peydayesh M, Sedighi A, AghaAskari H, Rohani M, Baniasadi M, Arkia S, Seyedi F, Khatami S. Copper Oxide NPs Greener Synthesis Using Tea and its Antifungal Efficiency on *Fusarium solani*. *Geomicrobiology J.*. 2019;**36**: 1. doi: 10.1080/01490451.2019.1621963.
22. Karthik K, Pradeeswari K, Mohan Kumar R, Murugesan R. Microwave-assisted V2O5 nanoflowers for efficient lithium-ion battery. *Mat Res Innovations*. 2019;**23**:1-5. doi: doi.org/10.1080/14328917.2019.1618044.
23. Khan AU, Yuan Q, Khan ZUH, Ahmad A, Khan FU, Tahir K, *et al*. An eco-benign synthesis of AgNPs using aqueous extract of Longan fruit peel: Antiproliferative response against human breast cancer cell line MCF-7, antioxidant and photocatalytic deprivation of methylene blue. *J Photochem Photobiol B*. 2018;**183**:367-373. doi: 10.1016/j.jphotobiol.2018.05.007.
24. Miri A, Sarani M. Biosynthesis, characterization and cytotoxic activity of CeO_2 NPs. *Ceram Int*. 2018;**44**(11):12642-12647. doi: 10.1016/j.ceramint.2018.04.063
25. Miri A, Sarani M. Biological studies of synthesized silver NPs using *Prosopis farcta*. *Mol Biol Rep*. 2018;**45**(6):1621-1626. doi: 10.1007/s11033-018-4299-0
26. Jamdagni P, Rana JS, Khatri P, Nehra K. Comparative account of antifungal activity of green and chemically synthesized Zinc Oxide NPs in combination with agricultural fungicides. *Int J Nano Dim*. 2018;**9**(2):198-208. doi: 10.1016/j.ijbiomac.2017.08.149.
27. Minhas FT, Arslan G, Gubbuk IH, Akkoz C, Ozturk BY, Asıkkutlu B, *et al*. Evaluation of antibacterial properties on polysulfone composite membranes using synthesized biogenic silver NPs with *Ulva compressa* (L.) Kütz. and *Cladophora glomerata* (L.) Kütz. extracts. *Int J Biol Macromol.*

2018;**107**:157-165. doi: 10.1016/j.ijbiomac.2017.08.149.
28. Tahir K, Ahmad A, Li B, Khan AU, Nazir S, Khan S, *et al*. Preparation, characterization and an efficient photocatalytic activity of Au/TiO2 nanocomposite prepared by green deposition method. *Mat Let.* 2016;**178**:56-59. doi: 10.1016/j.matlet.2016.04.176.
29. Khan FU, Chen Y, Khan NU, Khan ZUH, Khan AU, Ahmad A, *et al*. Antioxidant and catalytic applications of silver NPs using Dimocarpus longan seed extract as a reducing and stabilizing agent. *J Photochem Photobiol B.* 2016;**164**:344-351. doi: 10.1016/j.jphotobiol.2016.09.042.
30. Tahir K, Nazir S, Ahmad A, Li B, Ali Shah SA, Khan AU, *et al*. Biodirected synthesis of palladium NPs using *Phoenix dactylifera* leaves extract and their size dependent biomedical and catalytic applications. *RSC Adv*. 2016;**6**(89):85903-16. doi: 10.1039/C6RA11409A.
31. Ahmad A, Syed F, Shah A, Khan Z, Tahir K, Khan AU, *et al*. Silver and gold NPs from Sargentodoxa cuneata: synthesis, characterization and antileishmanial activity. *RSC Adv*. 2015;**5**(90):73793-806. doi: 10.1039/C5RA13206A.
32. Khatami M, Alijani HQ, Heli H, Sharifi I. Rectangular shaped zinc oxide NPs: Green synthesis by Stevia and its biomedical efficiency. *Ceram Int.* 2018;**44**(13):15596-15602. doi:10.1016/j.ceramint.2018.05.224.
33. Nagajyothi PC, Sreekanth TVM, Tettey CO, Jun YI, Mook SH. Characterization, antibacterial, antioxidant, and cytotoxic activities of ZnO NPs using *Coptidis Rhizoma*. *Bioorg Med Chem Lett.* 2014;**24**(17):4298-4303. doi: 10.1016/j.bmcl.2014.07.023.
34. Kim K-M, Choi M-H, Lee J-K, Jeong J, Kim Y-R, Kim M-K, *et al*. Physicochemical properties of surface charge-modified ZnO NPs with different particle sizes. Int J Nanomedicine. 2014;**9**(Suppl 2):41-56. doi: 10.2147/IJN.S57923.
35. Birla S, Tiwari V, Gade A, Ingle A, Yadav A, Rai M. Fabrication of silver NPs by Phoma glomerata and its combined effect against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Lett Appl Microbiol.* 2009;**48**(2):173-179. doi: 10.1111/j.1472-765X.2008.02510.x.
36. Rai M, Duran N. Metal NPs in microbiology: *Springer Science & Business Media;* 2011. doi: 10.1007/978-3-642-18312-6.
37. Delavari M, Dalimi A, Ghaffarifar F, Sadraei J. *In Vitro* Study on Cytotoxic Effects of ZnO NPs on Promastigote and Amastigote Forms of *Leishmania major* (MRHO/IR/75/ER). *Iran J Parasitol.* 2014;**9**(1):6-13. PMCID: PMC4289881. doi: 10.1049/iet-nbt.2018.5076.
38. Sumaira G, Afridi MS, Hashmi SS, Ali GS, Zia M, Abbasi BH. Comparative antileishmanial efficacy of the biosynthesised ZnO NPs from *genus Verbena*. *IET nanobiotech*. 2018;**12**(8):1067-1073. doi: 10.1049/iet-nbt.2018.5076.