Biosynthesis of spinel nickel ferrite nanowhiskers and their biomedical applications

Hajar Q. Alijani1,2, Siavash Iravani3, Shahram Pourseyedi1,2*, Masoud Torkzadeh-Mahani4, Mahmood Barani5 & Mehrdad Khatami6,7,8*

Greener methods for the synthesis of various nanostructures with well-organized characteristics and biomedical applicability have demonstrated several advantages, including simplicity, low toxicity, cost-effectiveness, and eco-friendliness. Spinel nickel ferrite (NiFe$_2$O$_4$) nanowhiskers with rod-like structures were synthesized using a simple and green method; these nanostructures were evaluated by X-ray diffraction analysis, transmission electron microscopy, scanning electron microscopy, and X-ray energy diffraction spectroscopy. Additionally, the prepared nanowhiskers could significantly reduce the survival of *Leishmania major* promastigotes, at a concentration of 500 μg/mL; the survival of promastigotes was reduced to ≃ 26%. According to the results obtained from MTT test (in vitro), it can be proposed that further studies should be conducted to evaluate anti-leishmaniasis activity of these types of nanowhiskers in animal models.

The nanowhiskers with unique shape, electrical, optical, magnetic, and surface properties have shown attractive clinical and biomedical potentials4,5. Typically, the production of different nanostructures with well-organized morphologies and sizes is highly demanded by researchers and scientists due to their unique applications and properties3–7.

Inorganic nanostructures with different mechanical and physical properties can be employed in different applications such as medicine, electronic device, sunscreens, military applications, photovoltaic cells, paints, catalysts, and among others8–16. Among nanostructures, nanofibers are defined as structures with an outer diameter below 1000 nm17,18. Nanowhisker is a type of nanofiber crystal with a diameter of less than 100 nm19. Nanowhiskers can have various applications in filtration20,21, food packaging22, diagnosis23, drug delivery24, gene delivery25–27, cancer therapy28 and cell scaffolding29.

In recent years, the mechanical properties and widespread applications of NiFe$_2$O$_4$ nanowhiskers have been demonstrated by researchers. Ferrites are ceramics made from a combination of iron oxide and divalent metals such as barium, strontium, lead, nickel, cobalt, among others30,31. Ferrites have wide applications in various biomedical32, catalytic27,33–35, wastewater36, extraction37, electrical38 fields.

For the fabrication of nanowhiskers, various synthesis approaches have been reported, including microwave39, carbo-thermal reduction40, and electrospinning41 techniques. However, one of the important drawbacks with these methods is the dependence on expensive equipment or energy consumption, which is directly or indirectly a threat to environmental health42. To solve this problem, it is necessary to discover environmentally friendly production methods for the synthesis of nanostructures43–47. Therefore, the green synthesis of nanostructures in various forms has been developed using plant extracts48. The application of plant extracts for synthesizing nanostructures is in accordance with the principles of green chemistry. This bio-based method has some important advantages of low toxicity and eco-friendliness, and for the creation of nanostructures, plant extracts act as natural reducing49 and stabilizing agents50–52.

---

1Department of Biotechnology, Shahid Bahonar University of Kerman, Kerman, Iran. 2Research and Technology Institute of Plant Production (RTIPPP), Shahid Bahonar University of Kerman, Kerman, Iran. 3Faculty of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran. 4Biotechnology Department, Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman, Iran. 5Medical Mycology and Bacteriology Research Center, Kerman University of Medical Sciences, 7616913555 Kerman, Iran. 6Noncommunicable Diseases Research Center, Bam University of Medical Sciences, Bam, Iran. 7Department of Medical Biotechnology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran. 8Cell Therapy and Regenerative Medicine Comprehensive Center, Kerman University of Medical Sciences, Kerman, Iran. *email: spseyedi@gmail.com; mehrdad7khatami@gmail.com
As far as we know, no studies have been conducted for greener biosynthesis of spinel ferrite nanowhiskers; therefore, we focused on greener synthesis of spinel ferrite nanowhiskers using rosemary extract. Rosemary (*Rosmarinus officinalis*) is a woody, evergreen and fragrant medicinal plant, which contains phytochemicals such as rosmarinic acid, betulinic acid, camphor, carnosic acid, caffeic acid, carnosol, and ursolic acid. The active ingredients in rosemary have suitable antioxidant, anti-inflammatory, and antibacterial effects.

Aqueous extract of rosemary was utilized to make NiFe₂O₄ nanostructures in a single step at pH 7. X-ray powder diffraction (XRD), high-resolution transmission electron microscopy (HR-TEM), scanning electron microscopy (SEM), and energy-dispersive X-ray spectroscopy (EDX) techniques were employed to characterize these nanostructures. Additionally, antiparasitic activities of these nanostructures against *Leishmania major* were evaluated, in vitro.

**Results**

**Characterization of NiFe₂O₄ nanowhiskers.** The crystal and fuzzy structure of the synthesized nickel-ferrite nanostructures in the range 10–70° is shown in Fig. 1. The peaks observed at 2θ correspond to the reverse spinel structure of nickel-ferrite nanostructures. The diffraction peaks correspond to lattice plane plates (220), (311), (222), (400), (422), (511), and (440)⁵³.

The structure of nickel-ferrite nanostructures is shown in HR-TEM image (Fig. 2, scale bar: 100 nm). According to the images, the surface of the synthesized nanostructures was smooth and even. The nanostructures were needle-like filaments (whiskers). Each hair-like strand has grown significantly in its longitudinal direction. Due to the presence of an interplate space of at least 10 nm, no aggregation or agglomeration of particles could be detected between hair-like strands such as NiFe₂O₄ nanowhiskers with soft surfaces. The size of these particles was less than 10 nm in width and more than 100 nm in length.

Figure 3a shows the surface morphology and number of constituents originated from NiFe₂O₄ nanowhiskers. As shown in SEM image, NiFe₂O₄ nanostructures are filamentous. The presence of nickel, oxygen and iron in the structure of the synthesized nanostructure was confirmed by EDX spectrum (Fig. 3b). EDX spectra demonstrated element ratios of Ni, Fe and O are 12, 24 and 48%, respectively.

**Efficacy evaluation of nickel-ferrite nanowhiskers on L. major.** The effectiveness of nickel-ferrite nanowhiskers with different concentrations for 48 h was evaluated based on MTT assay against *L. major* promastigotes (Fig. 4). Based on the obtained results, the survival rate of parasitic promastigotes was considerably lowered, when the concentration of nanostructures was increased. By applying nickel-ferrite nanowhiskers (with a concentration of 500 μg/ml), the survival of promastigotes was reduced to ≃ 26%, accordingly.

Nickel-ferrite nanowhiskers in a dose-dependent manner reduced the survival rate of *L. major* promastigotes, that when compared to the control group, this difference was statistically significant (p < 0.05). In MTT assay, IC₅₀ on *L. major* promastigotes was about 100 μg/ml.

**Discussion**

Nickel-ferrite nanowhiskers were synthesized in one step using rosemary phenolic extract at minimal cost (Fig. 5).
The cubic spinel structure of these spherical particles was identified by XRD analysis. Long strands of hair could be detected with a distinct morphologically margin of the whisker in HR-TEM.

Leishmaniasis is one of the most important infectious diseases in the world with 30,000 deaths annually\(^5\), which leads to the death by damaging macrophage cells, spleen, bone marrow, and liver. With the advancement of technology and the inadequacy of current therapeutic drugs, the application of nanostructures with unique properties and plant extracts with antimicrobial activities has recently attracted the attention of researchers\(^5\). Today, various nanostructures with different structures, suitable permeability, targeting properties, low toxicity, lack of resistance, high stability, and cost-effectiveness properties are widely deployed in the treatment of different diseases\(^5\). The transfer of amphotericin to glycine polymer-coated iron oxide nanoparticles could significantly reduce Leishmania parasite in the spleen\(^5\). Nickel oxide nanoparticles have anti-leishmaniasis activity against amastigotes and promastigotes of *Leishmania tropica* parasite\(^5\). But to the best of our knowledge, there are no studies about leishmanicidal activity of nickel-ferrite nanowhiskers against *L. major* promastigotes.

**Materials and methods**

**Greener synthesis of NiFe\(_2\)O\(_4\) nanowhiskers.** Rosemary leaves were collected from Kerman University Garden, Kerman, Iran. The rosemary leaves were collected in accordance with applicable institutional (Kerman University), national, and international rules and legislation. It was verified by the Iranian Botanical Survey, whose voucher specimen number was 1400/1 deposited at the Department Pharmacognosy, Kerman University.
Fresh rosemary leaves were picked from the plant at the flowering stage. The leaves were disinfected using sodium hypochlorite for 3 min, and were washed 5 times with sterile deionized water and dried at 27 °C. 100 g of fresh rosemary leaves were warmed for one hour at 80 °C in 500 ml deionized water. In the next step, the mixture of leaves and deionized water was allowed to stand for one hour at room temperature and finally, filter paper was employed to separate the extract. To synthesize nickel-ferrite nanowhiskers, 1.7 g of iron (III) chloride (FeCl₃.6H₂O, 98%, Merck) was dissolved on a heater at a temperature of 65–70 °C with 100 ml of aqueous rosemary extract by a strainer. 0.4 g of nickel chloride (II) (NiCl₂.6H₂O, 99%, Merck) was added to the above mixture and dissolved at the same temperature by a strainer. Then, one molar solution of sodium carbonate (Na₂CO₃ anhydrous, ≥ 99.5%, Sigma-Aldrich) was added dropwise to bring the pH of the mixture to 10. Then, the mixture was stirred for about 3 h at 65–70 °C. The resulting nanostructures were separated through centrifuging, and then were washed with ethanol-deionized water and deionized water. Finally, these nanostructures were dried for 16 h at 60 degrees Celsius in an electric oven and ground into a soft powder.

**Nickel-ferrite nanowhisker characterization.** The crystal structure, morphology, and weight percentage of NiFe₂O₄ nanowhiskers were studied using XRD (X’PertPro, Panalytical; with Cu lamp), HRTEM (Sigma VP, ZEISS), and scanning electron microscope (SEM) coupled with energy-dispersive X-ray spectroscopy (EDX, TESCAN, Czech Republic).

**Toxicity evaluation of nickel-ferrite nanowhiskers against L. major.** The Center for Disease Control, Ministry of Health, in Iran, introduced Glucantime (meglumine antimoniate) as the chosen medicine for the treatment of all clinical types of leishmaniasis. Promastigotes of *L. major* were cultivated at 24 °C in a 25 ml flask with RPMI1640 (Roswell Park Memorial Institute media), 10% fetal bovine serum (FBS), and 2% penicillin and streptomycin. A colorimetric cell viability approach was deployed to quantify viable cells in micro-well plates to examine the impact of the produced nickel-ferrite nanowhiskers on *L. major* promastigotes. Tetrazolium has a positive charge and may easily permeate living cells, converting MTT from a soluble to an insoluble dye compound that this procedure called MTT assay. In brief, 100 µl of stationary phase promastigotes (5 × 10⁴) cells/ml were introduced to a 96-well tissue culture plate. Following that, 100 µl of nickel-ferrite nanowhiskers (1, 5, 10, 25, 50, 100, 200, and 500 µg/ml) were applied to each well and maintained for 48 h at 25 °C. Following the incubation period, 10 µl of MTT solution at concentration of 5 mg/ml was applied to each well and stored at 25 °C for 4 h. After that, cold isopropanol was utilized as a solvent for Formazan crystals, resulting in a purple colour. ELISA reader was employed to detect each well's absorption at 493 nm (BioTek-ELX800, Winooiski, Vermont, USA). Promastigotes were cultured in drug-free RPMI1640 medium as control sample, and culture
medium without promastigote and drug was also utilized as a pure sample. In SPSS software, the 50% inhibitory concentration or IC50 was computed for all concentrations studied using the Probit test.

**Conclusion**

In this study, nickel-ferrite nanowhiskers were eco-friendly synthesized using aqueous extract of rosemary. XRD, FESEM-EDAX, and HR-TEM evaluations confirmed the spinel and needle-like structures of the prepared nanostructures. Results obtained from in vitro studies revealed that the toxicity of nickel-ferrite nanowhiskers was improved against *L. major* promastigotes by increasing the concentration of nickel-ferrite nanowhiskers and treatment duration.

Received: 20 May 2021; Accepted: 18 August 2021
Published online: 31 August 2021

**References**

1. Fu, Z. *et al.* Shape control of Cu/ZnO core-shell nanocubes and related structures for localized surface plasmon resonance. *ACS Appl. Nano Mater.* **4**, 995–999 (2021).
2. Foroughi, M. M., Jahani, S. & Rajaei, M. Facile fabrication of 3D dandelion-like cobalt oxide nanoflowers and its functionalization in the first electrochemical sensing of oxymorphone: evaluation of kinetic parameters at the surface electrode. *J. Electrochem. Soc.* **166**, B1300–B1311. https://doi.org/10.1149/2.0511914jes (2019).
3. Nasrollahzadeh, M., Sajjadi, M., Iravani, S. & Varma, R. S. Trinmetallic nanoparticles: greener synthesis and their applications. *Nanomaterials* **10**, 1784 (2020).
4. Raeisi, M. *et al.* Magnetic cobalt oxide nanosheets: green synthesis and in vitro cytotoxicity. *Bioprocess Biosyst. Eng.* **44**, 1423–1432 (2021).
5. Khatami, M., Zafarnia, N., Bami, M. H., Sharifi, I. & Singh, H. Antifungal and antibacterial activity of densely dispersed silver nanospheres with homogeneity size which synthesized using chicory: an in vitro study. *J de Mycologie Médicale* **28**, 637–644 (2018).
6. Mirzaei, H. *et al.* Direct growth of ternary copper nickel cobalt oxide nanowires as binder-free electrode on carbon cloth for nonenzymatic glucose sensing. *Microchem. J.* **142**, 343–351. https://doi.org/10.1016/j.microc.2018.07.014 (2018).
7. Khatami, M. *et al.* Calcium carbonate nanowires: greener biosynthesis and their leishmanicidal activity. *RSC Adv.* **10**, 38063–38068. https://doi.org/10.1039/D0RA04503A (2020).
8. Dong, P. *et al.* Controllable synthesis of exceptionally small-sized superparamagnetic magnetite nanoparticles for ultrasensitive MR imaging and angiography. *J. Mater. Chem. B* **9**, 958–968 (2021).
9. Wang, Z. *et al.* Large-scale one-pot synthesis of water-soluble and biocompatible upconversion nanoparticles for dual-modal imaging. *Colloids Surf B Biointerfaces* **198**, 111480 (2021).
10. Chen, Z. et al. Fabrication of cellulose paper containing zeolitic imidazolate framework and its application in removal of anionic dye from aqueous solution. BioResources 16, 2644–2654 (2021).
11. Hesaraki, M. et al. Knowledge, attitude, practice and clinical recommendations of health care workers towards COVID-19: a systematic review. Review. Environm.l Health 35, 1–13 (2020).
12. Dai, Z., Guo, S., Gong, Y. & Wang, Z. Semiconductor flexoelectricity in graphite-doped SrTiO3 ceramics. Ceram. Int. 47, 6535–6539 (2021).
13. Safaei, M. et al. A review on metal-organic frameworks: Synthesis and applications. TrAC. Trend. Analytic. Chem. 118, 401–425 (2019).
14. Rabiei, N. et al. Diatoms with invaluable applications in nanotechnology, biotechnology, and biomedicine: recent advances. ACS Biomater. Sci. Eng. https://doi.org/10.1021/acsbiomaterials.1c00475 (2021).
15. Sargazi, S. et al. Synthesis, characterization, toxicity and morphology assessments of newly prepared microemulsion systems for delivery of valproic acid. J. Mol. Liq. 338, 116625. https://doi.org/10.1016/j.molliq.2021.116625 (2021).
16. Sabouri, Z., Akbari, A., Hosseini, H. A., Khatami, M. & Darroudi, M. Egg white-mediated green synthesis of NiO nanoparticles and study of their cytotoxicity and photocatalytic activity. Polyhedron 178, 114351. https://doi.org/10.1016/j.poly.2020.114351 (2020).
17. VillaVelázquez-Mendoza, C. I. et al. Simultaneous synthesis of β-Si3N4 nanoibers and pea-pods and hand-fan like Si2N2O nanostructures by the CVD method. Mater. Lett. 175, 139–142. https://doi.org/10.1016/j.matlet.2016.04.028 (2016).
18. Guo, J., Gao, J., Xiao, C., Chen, L. & Qian, L. Mechanochemical reactions of GaN-Al2O3 interface at the nanoasperity contact: roles of crystallographic polarity and ambient humidity. Friction 2021, 1–14 (2021).
19. Dolete, G., Ille, C. F., Nicoara, I. F., Vlăsceanu, G. M. & Grumeseacu, A. M. In Nanobiomaterials in Dentistry (ed Alexandru Mihai Grumeseacu) 27–47 (William Andrew Publishing, 2016).
20. Gopi, S., Kargl, R., Kleinschek, K. S., Pius, A. & Thomas, S. Chitin nanowhisker-Inspired electrospon PVDF membrane for enhanced oil-water separation. J. Environ. Manage. 228, 249–259 (2018).
21. Liu, M., Xue, Z., Zhang, H. & Li, Y. Dual-channel membrane capacitive deamination based on asymmetric ion adsorption for continuous water desalination. Electrochem. Commun. 125, 106974 (2021).
22. Haghighi, H. et al. Characterization of bio-nano composite films based on gelatin/polyvinyl alcohol blend reinforced with bacterial cellulose nanowhiskers for food packaging applications. Food Hydrocolloids 113, 106454 (2021).
23. Tang, H. et al. High-strength paper enhanced by chitin nanowhiskers and its potential bioassay applications. Int. J. Biol. Macromol. 150, 885–893 (2020).
24. Dash, R. & Ragauskas, A. J. Synthesis of a novel cellulose nanowhisker-based drug delivery system. RSC Adv. 2, 3403–3409 (2012).
25. Zou, Q., Xing, P., Wei, L. & Liu, B. Gene2vec: gene subsequence embedding for prediction of mammalian N6-methyladenosine RNA sites from mRNA. J. Mol. Liq. 338, 116625. https://doi.org/10.1016/j.molliq.2021.116625 (2021).
26. Zhao, H. et al. Comprehensive landscape of epigenetic-dysregulated IncRNAs reveals a profound role of enhancers in carcinogenesis in BC subtypes. Mol. Therapy-Nucleic Acids 23, 667–681 (2021).
27. Rahimi, S., Poormohammadi, A., Salmani, B., Ahmadian, M. & Rezaei, M. Comparing the photocatalytic process efficiency using batch and tubular reactors in removal of methylene blue dye and COD from simulated textile wastewater. Journal of Water Reuse and Desalination 6, 574–582. https://doi.org/10.2166/wrd.2016.190 (2016).
28. Xu, J. et al. UBQLN1 mediates sorafenib resistance through regulating mitochondrial biogenesis and ROS homeostasis by targeting PGC1β in hepatocellular carcinoma. Signal Transduct. Target. Ther. 6, 1–13 (2021).
29. Krishnan, V. et al. Vortex-aligned fullerene nanowhiskers as a scaffold for orienting cell growth. ACS Appl. Mater. Interfaces. 7, 15667–15673 (2015).
30. Vedrtnam, A., Kalauni, K., Dubey, S. & Kumar, A. A comprehensive study on structure, properties, synthesis and characterization of ferrites. J. Sol-Gel. Sci. Technol. 265, 29–44 (2019).
31. Ordoukhanian, J., Nezhadali, A. & Mehri, L. Electrosynthesis of Co, Ni and Zn ferrites nanoparticles in the presence of external magnetic field. Mater. Lett. 175, 139–142. https://doi.org/10.1016/j.matlet.2016.04.028 (2016).
32. Patil, S., Naik, H. B., Nagaraju, G., Viswanath, R. & Rashmi, S. Sugarcane juice mediated eco-friendly synthesis of visible light active zinc ferrite nanoparticles: application to degradation of mixed dyes and antibacterial activities. Mater. Chem. Phys. 212, 351–362 (2018).
33. Malakotian, M. et al. Protocol encompassing ultrasound/Fe3O4 nanoparticles/persulfate for the removal of tetracycline antibiotics from aqueous environments. Clean Technol. Environm. Policy 21, 1665–1674. https://doi.org/10.1007/s10098-019-01733-w (2019).
34. Seid-Mohammadi, A., Gh, A., Sammadi, K., Ahmadian, M. & Poormohammadi, A. Removal of humic acid from synthetic water using chitosan as coagulant aid in electrocoagulation process for Al and Fe electrodes. Mater. Today Proc. 37, 119–121 (2021).
35. Lao, X., Xu, X., Jiang, W., Liang, J. & Miao, I. Influences of Al metal and Al–Si alloys on in-situ synthesis of SiC nanowhiskers in porous Al2O3–SiC composites obtained by carbothermal reduction. J. Alloys Compd. 92, 1665–1674. https://doi.org/10.1007/s10098-019-01733-w (2019).
36. Raessi, M. et al. Barium carbonate nanostructures: Biosynthesis and their biomedical applications. Ceram. Int. https://doi.org/10.1016/j.ceramint.2021.01.256 (2021).
37. Gopi, S., Kargl, R., Kleinschek, K. S., Pius, A. & Thomas, S. Chitin nanowhisker-Inspired electrospon PVDF membrane for enhanced oil-water separation. J. Environ. Manage. 228, 249–259 (2018).
38. Hussain, K., Amin, N. & Arshad, M. I. Evaluation of structural, optical, dielectric, electrical, and magnetic properties of Ce3+ doped CaO. 25CoO. 25Fe2-xO4 spinel nano-ferrites. Ceramics Int. 47, 3401–3410 (2021).
39. Banerjee, K., Sarkar, P., Pradhan, S., Bandyopadhyay, S. K. & Chaudhuri, S. A perspective review on the future of bio-nano composites: a review. Mater. Today Proc. 37, 119–121 (2021).
40. Sun, M. et al. Effects of NaClO shock on MBR performance under continuous operating conditions. Environ. Sci.: Water Res. Technol. 7, 396–404 (2021).
41. Zhang, L. et al. Effect of Fe3+ on the sludge properties and microbial community structure in a lab-scale A2O process. Sci. Total Environ. 780, 146505 (2021).
42. Hamidian, K., Saberian, M. R., Miri, A., Sharifi, F. & Sarani, M. Doped and un-doped cerium oxide nanoparticles: biosynthesis, characterization, and cytotoxic study. Ceram. Int. 47, 13895–13902. https://doi.org/10.1016/j.ceramint.2021.01.256 (2021).
43. Mohammadnejad, R., Karimi, S., Irvani, S. & Varma, R. S. Plant-derived nanotubes: types and applications. Green Chem. 18, 20–52. https://doi.org/10.1039/C5GC01403D (2016).
44. Hamidian, K., Saberian, M. R., Miri, A., Sharifi, F. & Sarani, M. Doped and un-doped cerium oxide nanoparticles: biosynthesis, characterization, and cytotoxic study. Ceram. Int. 47, 13895–13902. https://doi.org/10.1016/j.ceramint.2021.01.256 (2021).
49. Azhdari, S. et al. Metallic SPIONP/AgNP synthesis using a novel natural source and their antifungal activities. RSC Adv. 10, 28737–28744 (2020).

50. Khatami, M. et al. Greener synthesis of rod shaped zinc oxide nanoparticles using lilium ledebourii tuber and evaluation of their leishmanicidal activity. Iran. J. Biotechnol. 18, e2196–e2196. https://doi.org/10.30498/IJB.2020.119481.2196 (2020).

51. Alinaghi Langari, A. et al. CeO2 foam-like nanostructure: biosynthesis and their efficient removal of hazardous dye. Bioprocess Biosyst. Eng. 44, 517–523. https://doi.org/10.1007/s00449-020-02464-9 (2021).

52. Khatami, M. et al. Simplification of gold nanoparticle synthesis with low cytotoxicity using a greener approach: opening up new possibilities. RSC Adv. 11, 3288–3294. https://doi.org/10.1039/D0RA08822F (2021).

53. Mirgorod, Y. A., Borshch, N. A., Fedosyuk, V. M. & Yurkov, G. Y. Magnetic properties of nickel ferrite nanoparticles prepared using flotation extraction. Inorg. Mater. 49, 109–114. https://doi.org/10.1134/S0020168512110064 (2013).

54. Nafari, A. et al. Nanoparticles: New agents toward treatment of leishmaniasis. Arasite epidemiology and control, 10, e00156 (2020).

55. Kumar, R. et al. Development of high efficacy peptide coated iron oxide nanoparticles encapsulated amphotericin B drug delivery system against visceral leishmaniasis. Mater. Sci. Eng., C 75, 1465–1471 (2017).

56. Iqbal, J. et al. Green synthesis and characterizations of Nickel oxide nanoparticles using leaf extract of Rhamnus virgata and their potential biological applications. Appl. Organometal. Chem. 33, e4950 (2019).

Acknowledgements
This research was carried out using the funding and support of Research and Technology Institute of Plant Production of Shahid Bahonar University of Kerman, Iran. Also was supported by the VEGA Agency under the Contract no. 2/0140/20 and Bam University of Medical Sciences (M. Khatami grant No. 1400/018), Electron microscopy at the Institute of Macromolecular Chemistry was supported through Grants 17-05007S (Czech Science Foundation) and POLYMAT LO1507 (Ministry of Education, Youth and Sports of the CR, program NPU I).

Author contributions
Pourseyedi, SH. Barani, M. and Khatami, M. wrote the main manuscript text and Iravani, S. and Alijani, H prepared figures 1-3. All authors reviewed the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
Correspondence and requests for materials should be addressed to S.P. or M.K.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2021