



Novel and simple approach using synthesized nickel nanoparticles to control blood-sucking parasites

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ABSTRACT

The present study was on assessment of the anti-parasitic activities of nickel nanoparticles (Ni NPs) against the larvae of cattle ticks *Rhipicephalus (Boophilus) microplus* and *Hyalomma anatolicum (a.) anatolicum* (Acari: Ixodidae), fourth instar larvae of *Anopheles subpictus*, *Culex quinquefasciatus* and *Culex gelidus* (Diptera: Culicidae). The metallic Ni NPs were synthesized by polyol process from Ni-hydrazine as precursor and Tween 80 as both the medium and the stabilizing reagent. The synthesized Ni NPs were characterized by Fourier transform infrared (FTIR) spectroscopy analysis which indicated the presence of Ni NPs. Synthesized Ni NPs showed the X-ray diffraction (XRD) peaks at 42.76°, 53.40°, and 76.44°, identified as 111, 220, and 200 reflections, respectively. Scanning electron microscopy (SEM) analysis of the synthesized Ni NPs clearly showed that the Ni NPs were spherical in shape with an average size of 150 nm. The Ni NPs showed maximum activity against the larvae of *R. (B.) microplus*, *H. a. anatolicum*, *A. subpictus*, *C. quinquefasciatus* and *C. gelidus* with LC₅₀ values of 10.17, 10.81, 4.93, 5.56 and 4.94 mg/L; *r*² values of 0.990, 0.993, 0.992, 0.950 and 0.988 and the efficacy of Ni-hydrazine complexes showed the LC₅₀ values of 20.35, 22.72, 8.29, 9.69 and 7.83 mg/L; *r*² values of 0.988, 0.986, 0.989, 0.944 and 0.978, respectively. The findings revealed that synthesized Ni NPs possess excellent larvicidal parasitic activity. To the best of our knowledge, this is the first report on larvicidal activity of blood feeding parasites using synthesized Ni NPs.

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1. Introduction

Ticks transmit a greater variety of infectious organisms than any other group of blood sucking arthropods

(Sonenshine et al., 2002). Ticks are responsible for severe losses due to tick worry, blood loss, hide damage, injection of toxins, and diseases transmitted by the parasite (Ducornez et al., 2005). *Rhipicephalus (Boophilus) microplus* is one of the most widely distributed tick species and constitutes a major problem for the cattle industry in tropical and subtropical regions of the world. The bovine tropical theileriosis transmitted by *Hyalomma anatolicum (a.) anatolicum* is an economically important disease of cross-breed

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cattle in India which causes heavy economic losses in terms of high morbidity, mortality, and reduced production in recovered animals. In India, around 10 million cattle are at risk for tropical theileriosis with an annual economic loss of US \$800 million (Brown, 1997). Continuous and indiscriminate use of acaricides have led to the selection of chemical resistant ticks along with contamination of the environment and animal products (Graf et al., 2004).

Diseases such as dengue fever, dengue hemorrhagic fever, Japanese encephalitis, malaria, and filariasis are transmitted by blood-feeding mosquitoes increasing in prevalence, particularly in tropical and subtropical zones. *Anopheles* is an important vector for the transmission of malaria (Gutiérrez et al., 2008) and *Culex* is known for filarial transmission in human and lumbar paralysis in cattle (Kwong-Chung et al., 2004). There may have been over 225 million malaria infections in tropical and subtropical countries in 2009, resulting 781,000 deaths (WHO, 2010).

Nanoparticles (NPs), generally considered as particles with a size of up to 100 nm which exhibit completely new or improved properties as compared to the larger particles of the bulk material as they are composed of based on specific characteristics such as size, distribution, and morphology (Willems and van den Wildenberg, 2005). Gong et al. (2011) reported the toxicity of nickel oxide NPs to algae and batch cultured *Chlorella vulgaris* on growth and morphological changes under NPs exposure. Ada et al. (2010) reported the cytotoxicity and apoptotic effects of nickel oxide NPs on human cervix epithelioid carcinoma cell line (HeLa). The early embryo-larval developmental toxicity of nickel to *Xenopus laevis*, *Bufo terrestris*, and *Gastrophryne carolinensis* was evaluated using a modified frog embryo teratogenesis assay *Xenopus* model (Fort et al., 2006).

Ni NP is a product with many new characteristics, which include a high level of surface energy, high magnetism, low melting point, high surface area and low burning point. Therefore, it can be widely used in modern industries as catalysts, sensors and electronic applications (Zhang et al., 2003; Sivulka, 2005). Ni NPs caused cytotoxicity and apoptosis in mouse epidermal JB6 cells (Zhao et al., 2009); the cytotoxic effects in leukemia cancer cells (Guo et al., 2008) and the toxicity and developmental defects of different sizes and shape Ni NPs in zebrafish were reported (Ispas et al., 2009). Ahamed (2011) reported that the Ni NPs have potential to induce cytotoxicity, oxidative stress and apoptosis (caspase-3 activity) in human lung epithelial A549 cells. Earlier investigators reported the cytotoxicity of Ni NPs in mouse epidermal JB6 cells and leukemia cancer cells (Guo et al., 2008; Zhao et al., 2009). Ispas et al. (2009) have reported that the effect of Ni NPs sized at 30, 60, and 100 nm as well as aggregated particle clusters of 60 nm having dendritic structures were delivered to zebrafish embryos to assess changes in mortality and developmental defects. Ni hydroxide nanoparticles, are increasingly used in the power and energy industries, induce significant inflammatory responses in mouse lungs in a dose-dependent manner (Gillespie et al., 2010).

Pour-on application method of Ni NPs may be followed on the body surface of vertebrates. The pour-on application method was chosen because it is easy to carry out, reduced environmental pollution and practical, especially where no

dip tanks are available or when just a few animals need to be treated (Anon, 2004). Pour-on treatment required the application of the material used (discriminating doses) along the backline of the animal using a graduated squeeze bottle, where the liquid was dispersed over the animal's body surface, with the exception of the head, to contact parasites (Zahir and Rahuman, 2012). The present findings reveal the potential of Ni NPs to target ectoparasites such as ticks and mosquitoes and suggest the possibility for applications of the Ni NPs in related clinical and biomedical areas.

The present study was designed to investigate toxic effect of Ni NPs against blood-sucking parasites. Furthermore the aim of the present study was to control blood-sucking parasites using synthesized nickel nanoparticles against *R. (B.) microplus*, *H. a. anatolicum*, *Anopheles subpictus*, *Culex quinquefasciatus* and *Culex gelidus*. In the present study, we reported that the nickel NPs would be useful in promoting research aiming at the development of new agent for acaricidal and mosquito larvicidal activity.

2. Materials and methods

2.1. Materials

Ni (CH₃COO)₂·2H₂O (nickel acetate), N₂H₄·2H₂O (hydrazine) and Na₂CO₃ (sodium carbonate) were purchased from Aldrich (UK) and used without further purification.

2.2. Synthesis of Ni NPs

In the present synthesis, spherical Ni NPs were prepared by the thermal decomposition of Ni-hydrazine complexes and subsequent reduction of Ni ions was synthesized with some modification and as per the method of Libor and Zhang (2009). Nickel acetate (0.1 mol) aqueous solution was heated to 50 °C and then hydrazine (N₂H₄·2H₂O, 0.25 mol) was added to the solution with vigorous stirring. The solution was then heated to 65 °C, which resulted in light violet precipitate. The solution was cooled to 50 °C, and an aqueous solution of sodium carbonate (0.3 mol) was added to it. To obtain the spherical nanoparticles, the solution was again heated to 55 °C and remained for 1 h. The precipitated particles were retrieved by centrifugation. The yield of the overall synthesis was 60% based on the amount of Ni acetate. The formed black Ni precipitate was finally washed five times with distilled water and dried at 40 °C in oven overnight.

2.3. Characterization of Ni NPs

Infrared spectra were recorded in the range 400–4000 cm⁻¹ at 2 cm⁻¹ resolution using Bruker optics, FTIR spectrometer, Germany. For X-ray diffraction studies, dried nanoparticles were coated on XRD grid and the spectra was recorded by using Phillips PW 1830 instrument operated at a voltage of 40 kV and a current of 30 mA with Cu Kα₁ radiation. X-ray diffraction spectrum of the Ni NPs exhibited 2θ values corresponding to the Ni nanocrystal. For electron microscopic studies, 25 μl of sample was

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