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Vermifugal Activity of Biofabricated Silver Nanoparticles

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Abstract

Nanotechnology is an area of extensive research in recent years. Applications of noble metals have increased throughout human civilization and noble metal nanoparticles have benefited multiple sectors using their nano-scale applications. The possibility of using plants in the deliberate synthesis of nanoparticles is a recent phenomenon. Helminth infections are among the most common infections in humans, affecting a large population of the World. As per WHO, only few drugs are frequently used in the treatment of these parasite infections. Preliminary investigations were carried out to assess the vermifugal actvity of biological silver nanoparticles in-vitro. Albendazole and piperazine citrate were used as standard reference drugs and normal saline as control. Various concentrations of silver nanoparticles and aqueous extract of Hibiscus rosasinensis were tested as per standard protocols. Silver nanoparticles exhibited significant antihelminthic activity as compared to standard.

Keywords: Silver nanoparticles, antihelminthic activity, pheretima posthuma, mebendazole, piperazine citrate.

Introduction

"Nanotechnology" is the application of science to control matter at the molecular level. Development, characterization, and exploitation of nanophase materials are all fundamental to the anticipated nanotechnology revolution. Nanoparticles, sized between 1 and 100 nm, is gaining impetus in the present century as they posses defined chemical, optical and mechanical properties. This is mainly due to the possibility of modifying the physical properties of these systems through the control of the system size. The potential benefits of nanoparticles in biomedical and industrial applications for human health and environment are now well documented[1].

Among the various nanoparticles, metal nanoparticles assume special importance because they are easier ans cheaper to synthesize and are the most promising in applications. Silver nanoparticles are particularly interesting because they have unique optical, electrical and biological properties which offers a number of exciting potential applications in various fields including catalysis, electronics, and biology. They possess an excellent biocompatibility and low toxicity. Silver nanoparticles can be synthesized through an array of methods like spark discharging, electrochemical reduction, solution irradiation and cryochemical synthesis[2]. Chemical reduction is the most frequently applied method and silver nitrate ($AgNO_3$) is the predominant precursor used in the synthesis of silver nanoparticle due to its high chemical stability and low cost[3].

Nature constitutes a rich source of inspiring concepts for the design and production of materials. Integration of green chemistry principles to nanotechnology is one of the key issues in nanoscience research. Biological synthesis of nanoparticle relies on living cells as a machine that operates at the nano level and generates targeted materials at very high efficiency. The development of reliable green process for the synthesis of bioactive nanoparticles is of fundamental importance in the advancement of current nanotechnology research as they are significant components of innovative nanomedical devices to be applied to diverse medical and industrial fields for the betterment of mankind. A green approach for the synthesis of bioactive silver nanoparticles by an environment-friendly, simple, cost-effective, stable and reproducible aqueous room temperature method using the leaf extract of Hibiscus rosasinensis has been reported[4].

Helminthiasis is one among the most widespread macroparasitic infection with parasitic worms such as pinworm, roundworm, or tapeworm in humans and animals and represent a significant socio-economic concern[5]. WHO estimates that more than two billion people are affected with parasitic helminth infections worldwide[6].

Typically, the worms reside in the gastrointestinal tract but may also burrow into the liver or other organs. Infestation can cause morbidity, and sometimes death, by compromising nutritional status, affecting cognitive processes, inducing tissue reactions, and provoking intestinal obstruction or rectal prolapse[7]. Gastrointestinal nematodes adversely affect

livestock productivity, decrease in resistance to other diseases and cause severe mortality leading to heavy economic losses[8].

Anthelmintic drugs have been used either prophylactically or curatively to control these parasites. Albendazole, is a benzimidazole carbamate highly effective in ascariasis, intestinal capillariasis, entrobiasis, trichuriasis, and hookworm infections[9]. As a vermicidal, albendazole causes degenerative alterations in the tegument and intestinal cells of the worm by binding to the colchicine sensitive site of tubulin, thus inhibiting its polymerization or assembly into microtubules. The loss of the cytoplasmic microtubules leads to impaired uptake of glucose by the larval and adult stages of the susceptible parasites, and depletes their glycogen stores. Degenerative changes lead to decreased production of adenosine triphosphate (ATP), which is the energy required for the survival of the helminth. Due to diminished energy production, the parasite is immobilized and eventually dies[10]. Piperazine citrate is a water soluble, recognized therapeutic antihelminthic organic compound containing piperazine as the functional group. Which paralyses the parasites, allowing the host body to expel the worms. During treatment, worms whose phenotype renders them susceptible to the drug are killed. Worms that are resistant survive and pass on their "resistance" genes. The emergence of resistance to all the anthelmintics that are used currently[11,12] warrants the search for newer antihelmintic molecules for future use[12, 13].

Hibiscus rosasinensis: Hibiscus rosasinensis Linn (Malvaceae) is a medicinal herb usually used effectively in native medicines against hypertension, pyrexia and liver disorder, for the control of dysfunctional uterine bleeding and as an oral contraceptive. The plant is considered emollient, emmenagogue, anodyne, expectorant, refrigereant, anti-infectious, antihelminthic, anti inflammatory, diuretic, antipyretic, hypotensive, anti spasmodic, astringent, purgative, antidote for poison, constipating, hypoglycaemic, aphrodisiac, it is used to treat dandruff and stimulate hair growth. Hibiscus rosasinensis contains numerous compound including quercetin, glycosides, thiamine, riboflavin, niacin, carotene, anthocyanin, antho cyaniding, calcium oxalate, ascorbic acid, malvalic acid, gentisic acid, margaric acid and lauric acid. Flowers contain flavanoids and proanthocyanidins and polysaccharides. Certain phenolic compounds present attenuate neuronal death induced by oxidative stress. The present study is a preliminary evaluation of the vermifugal activity of silver nanoparticles synthesized from aqueous extract of Hibiscus rosasinensis.

Material and Methods

Drugs and chemicals used: The following drugs and chemicals were used. Piperazine citrate (Glaxo Smithkline) and Albendazole (Pfizer, Mumbai) were used as reference standards. Silver nitrate ($AgNO_3$) cryst. extra pure was procured from Merck, Germany. Normal saline, double distilled water and de-mineralized water were used throughout the experiments.

Synthesis of silver nanoparticles: Fresh leaves of *Hibiscus rosasinensis* were thoroughly washed, finely cut and stirred with 200 ml of de-mineralized water and filtered to obtain the extract which was used as the reducing agent and stabilizer. The aqueous leaf extract was challenged with 1mM Silver nitrate ($AgNO_3$) solution at ambient temperature and optimal conditions to obtain the silver nanoparticles. Double distilled water was used throughout the experiments. The bioreduction was monitored by periodic sampling of aliquots of the aqueous component and measuring the UV– Visible spectra of the solution in a Varian, Cary 5000 model with a limiting resolution of 0.05nm. The synthesized nanoparticles were characterized by UV-Vis Spectroscopic analysis, Photoluminiscence, High Resolution Scanning Electron Microscopy (HR-SEM) JEOL Model JSM - 6390LV, X-ray diffraction (XRD) on Bruker AXS D8 and Fourier Transform Infrared (FTIR) spectroscopy on a Bruker III, 400MHz model.

Experimental model: The common Indian adult earthworm, Pheretima posthuma, (Oligochaeta), found in wet soils rich in organic manure served as the test organisms. Pheretima posthuma has significance in antihelminthic screenings due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings[14, 15] and easy availability. Prior to experiment, Pheretima posthuma were collected from moist soil and kept for several days in darkness in cow dung at a constant temperature of 25°C in a climate-controlled room. The earthworms of 4- 6 cm in length and 0.2-0.3 cm in width were used in the experimental protocol. The earthworms ranged in live (wet) mass between 260 and 340 mg. The earthworms were washed with normal saline to remove all fecal matter and suspended in phosphate buffered saline.

Evaluation of anthelmintic activity: Adult motility in-vitro assay was conducted on mature live adult earthworms, Pheretima posthuma as a pre-screen means for anthelmintic activity according to the method of Ajaiyeoba[16]. Albendazole (20mg/ml) and piperazine citrate (10mg/ml) were used as reference drugs and phosphate buffered saline served as control. All the test solutions and standard drug solutions were prepared freshly before starting the experiment. Nine groups of six worms each, randomly selected, of approximately equal size were exposed in triplicate to each of the following treatments in separate Petri dishes containing 50 ml of different concentrations of aqueous extracts of Hibiscus rosasinensis (25, 50, 75 and 100 mg/ml), silver nanoparticles (250, 750 and 1000 ppm) and the standard drugs at room temperature. The motility of the worms were used as the criterion for the antihelminthic activity. The mean time of onset of paralysis and mortality of

the worm was observed and recorded in minutes. The paralysis time was recorded when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms was recorded when worms neither moved while shaken vigorously nor when dipped in luke warm saline water with fading away of the body colors. Finally, the treated worms were kept for 30 mins ine luke warm fresh phosphate buffered saline to observe the revivial of mortality for a certain period of time.

Statistical Analysis: All the grouped data were expressed as mean + SEM. The results were analyzed for statistical significance using one-way ANOVA followed by Dunnet's test. $P<0.05$ were considered significant.

Results and Discussion

Hibiscus rosasinensis leaf extract was found to be a successful agent in the biological synthesis of silver nanoparticles. The colour of the reaction medium changed rapidly from light green to yellowish brown after bioreduction. Figure 1. UV–visible spectroscopy is an important technique to ascertain the formation and stability of metal nanoparticles in aqueous solution. It is well known that silver nanoparticles exhibit yellowish brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles. Figure 2. The surface plasmon resonance peak in the UV-Vis absorption spectra of the silver nanoparticles synthesised by biological reduction showed an absorption peak at 417 nm.

figure 3. High resolution scanning electron microscopic analysis provided information on the morphology and size of the nanoparticles was found to be on an average of 35nm. Figure 4. Data obtained from the assay observations gives substantial evidence that silver nanoparticles could induce a acute/ lethal effect on the physiological function of Pheretima posthuma in vitro. Figure 5. After short-term exposure (8-10 mins) of Pheretima posthuma to silver nanoparticles in de-mineralized water, the earthworm showed increased sluggishness and lack of movements The noticeable difference in response of the test organism between the silver nanoparticle group and the aqueous extract group in the experimental setup could be ascribed to an interference with some vital processes of the particle conveyed by the nanodimension. Figure 6.

From the data obtained, it was observed that the silver nanoparticles exhibited significant vermifugal potential and demonstrated paralysis as well as death of worms in a time comparable to the standard drugs used. Control worms were alive throughout the experiment. The highest concentration of silver nanoparticle solution (1000 ppm) produced paralytic effect much earlier and the time to death was shorter.

Literature cites that the synthetic antihelmintic, piperazine citrate, by increasing the chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis which results in expulsion of the worm by peristalsis[17].

Albendazole acts by blocking glucose uptake and depletion of glycogen stores in the parasite. The antihelminthic activity of the aqueous extract of Hibiscus rosasinensis in a dose dependent manner also gave satisfying results, taking shortest time for paralysis and death with 100 mg/ml concentration. This could be attributed to the flavanoid and other phenolic phytochemicals present in the plant extract. Synthetic polyphenolic anthelmintics are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation[18]. The present study is, to the best of our knowledge, the first, to report the vermifugal efficiency of biologically synthesized silver nanoparticles in Pheretima posthuma.

The toxic action of the nanoparticles can be linked to a chemical effect and/ or stress or stimuli caused by the peculiar physical characteristics of the nanostate[19, 20].

Figure-1
Hibiscus rosasinensis leaves used for bioreduction

Figure 2
Aqueous extract of Hibiscus rosasinensis leaves and the synthesized silver nanoparticle solution

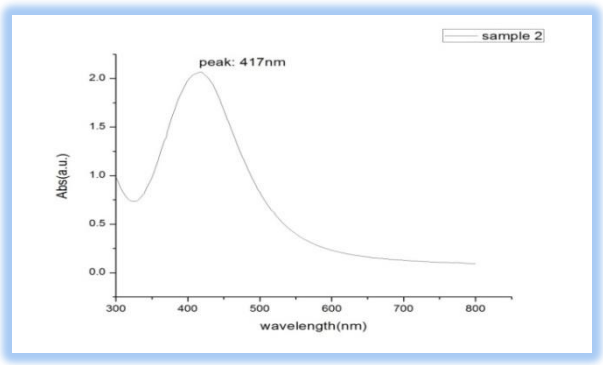

Figure 3
UV-Visible spectra of silver nanoparticles from Hibiscus rosasinensis

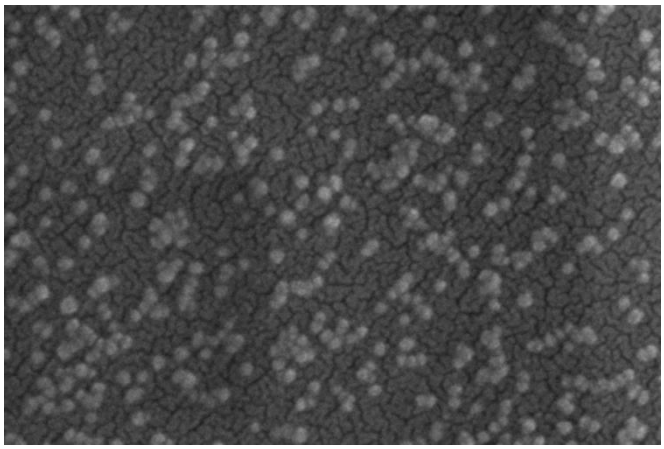

Figure - 4
Scanning Electron Microscope image of the synthesized silver nanoparticles

Figure - 5
In-vitro evaluation of effect of silver nanoparticles on Pheretima posthuma

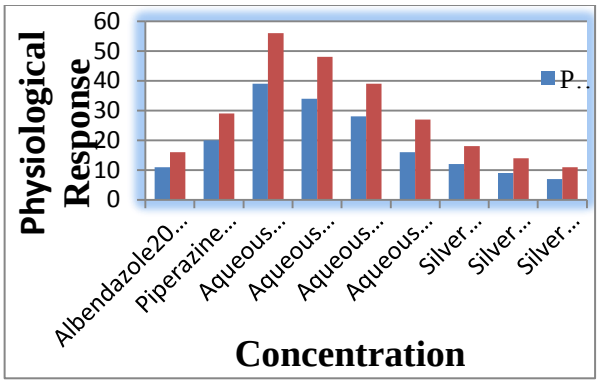

Figure-6
Vermifugal activity of silver nanoparticle and aqueous extract of Hibiscus rosasinensis on Pheretima posthuma compared to standard drugs

Conclusion

The potential uses and benefits of nanotechnology are enormous. Silver nanoparticles, with their extraordinary properties, have diverse *in vitro* and *in vivo* biological applications. Silver nanoparticles, obtained by a green chemistry synthetic route exhibited significant vermigufal activity in vitro. These findings constitute only a preliminary insight into the comprehension of the biophysico-chemical interactions of nanomaterials with the biological entities in the experimental model. More targeted approaches and on-going research into the capabilities and possibilities for silver colloids could yield novel marketable products of use to man-kind.

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