Potential applications of inorganic silver nanoparticles (AgNPs) in parasitic diseases

Review Article

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ABSTRACT

Parasitic diseases are globally distributed, leading to considerable morbidity and mortality especially in low and middle-income countries. The current treatment strategies of parasitic infections face hard challenges due to emergence of drug resistance and intolerable side effects. Therefore, the development of novel, safe and effective anti-parasitic agents is urgently required. In this context, nanomaterials represent an efficient tool to overcome the barriers observed in several conventional therapeutics. Nanotechnology has efficiently provided tools enabling structural modifications in chemical compounds, empowering their highly desirable selectivity and specificity. Among them, metal nanoparticles (NPs) have been significantly utilized as an alternative therapeutic approach and/or efficient delivery system in a wide range of parasitic diseases. Due to its extraordinary range of bactericidal properties and therapeutic abilities, AgNPs have become a part of medical management of various diseases. Moreover, a number of plant species successfully employed in AgNPs green synthesis, are ecofriendly efficient alternatives to chemically synthesized NPs. The current review aims to shed light on AgNPs regarding their physiochemical properties, synthesis methods, and potential applications in diagnosis, treatment, and protection against parasitic infections. Concerning the last issue, their insecticidal activities paved the way for prevention of transmission of vector borne parasitic diseases.

Keywords: AgNPs, anthelmintic, antiprotozoal, chemical synthesis, diagnosis, green synthesis, insecticidal, *in vitro*, *in vivo*.

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INTRODUCTION

Bearing in mind the extent of scientific discoveries and inventions throughout human existence on earth, the advent of nanotechnology is a relatively recent advancement. The last three decades witnessed the invention of nanotechnology with extensive commercial applications. Notably, NPs are materials with distinctive properties, downscaled to the level of individual atoms and molecules, generally reaching a size of 1-100 nm. Potential usages for NPs comprise industrial, agricultural, and medicinal purposes. Although NPs possess a chemical constitution that is identical to the parent material, physical properties of color, solubility, stability, magnetic and thermodynamic properties, and other attributes may differ[1]. Governed by their physical and chemical features, nanomaterials (NMs) are categorized into four major classes, carbon, metallic, organic and nanocomposites. Metallic NMs include gold, silver, selenium, platinum; and metal oxides such as zinc oxide, iron oxide, copper oxide, titanium dioxide and magnesium oxide[2].

Gold and silver NMs advanced molecular diagnostics and introduced radical new treatments[3]. The two NMs possess excellent properties as anticancer, antimicrobial, antidiarrheal and antifungal medications, and are effective in wound healing. Due to their remarkable disinfectant properties, AgNPs proved highly effective as sterilizing agents. Other applications of AgNPs include diagnostic (biological tags in biosensors, assays, and quantitative detection), optical (metal-enhanced fluorescence and surface-enhanced Raman scattering), conductive (conductive inks, pastes, and fillers), and household (pesticides and wastewater treatment)[4].

Their use in diagnostic instruments is based on their proficiency to exhibit a visible surface plasmon resonance. This is induced when a light beam is directed at a particular angle on the metal surface. The combined oscillation of electrons at the metal surface, provides them with an illustrative yellow color in aqueous solutions[5]. Furthermore, AgNPs are distinguished by their reactivity toward thiolated ligands, offering the discriminate functionalization of surface substrates to detect particular biological or synthetic targets[6]. They possess unique physical and chemical properties that distinguish their advantages over other metallic NPs. These advantages include the likelihood of high-scale construction, possession of long-standing stability, provision of well-ordered drug delivery, and the probability of easy powder-formulation through freeze-drying and lyophilization[7].

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Parasitic diseases are one of the world's most devastating and prevalent infections. Some of the current specific therapeutics of parasitic infections are limited by development of pathogen resistance. This entailed increase of administration dose and frequency with subsequent increased adverse effects up to toxicity. Furthermore, the intracellular location of some parasites hinders the access of antimicrobial drugs[8]. Hence, there is a pressing need for development of novel, safe and effective anti-parasitic agents and/or improvement of the delivery of the available drugs in order to enhance their bioavailability. In this context, NPs could represent an efficient way to overcome the barriers found in conventional therapeutics. The use of nano-delivery systems enables structural modifications in chemical compounds, empowering them with highly desirable selectivity and specificity[9].

The present review is intended to shed light on one of the important members of inorganic metal NPs, the "AgNPs", regarding the physiochemical properties, the different methods of synthesis, the diagnostic applications in parasitic infections, the *in vitro* and *in vivo* anti-parasitic activities, the drug-delivery properties and finally the preventive potentials. Different medically important parasites, affected by AgNPs are listed in this review. Plants that were successfully used in green synthesis of AgNPs are also listed. Selected articles were gathered from Medline (PubMed), Scopus, Science Direct, Web of Science and Google Scholar using the keywords: metal NPs, nanodrug, nano-medicine, inorganic, silver, green synthesis, anti-parasitic, parasitic infections, *Leishmania*, *Toxoplasma*, toxoplasmosis, *Trypanosoma*, *Plasmodium*, malaria, *G. lamblia*, *E. histolytica*, *Cyclospora, Cryptosporidium, Blastocystis Acanthamoeba, Naeglaria, Schistosoma, Fasciola*, hydatid cyst, *Trichinella, Anopheles, Aedes, Culex*, insecticidal, larvicidal. To collect precise literature data, a comprehensive search was carried out on all published and unpublished resources, including full texts, and abstracts published during the period from 1st January 2009 to 31th December 2021.

Physical and chemical properties

Cytotoxicity of AgNPs is governed by its physical and chemical properties. These include surface chemistry, particle morphology, constitution, size and distribution, as well as its reactivity in solution, coating/capping agents, agglomeration, dissolution rate, proficiency of ion release, and lastly the type of reducing agents used for synthesis[10-12]. The use of culture supernatants of various bacillus species as reducing agent synthesizes different spherical, rod, octagonal, hexagonal, triangle, or flower-like AgNPs shapes. Regarding the size of AgNPs, it was postulated that smaller sized particles could be more toxic than larger ones due to larger surface area[13]. Additionally, the NP' shape is equally important for the determination of toxicity, where the smaller size and truncated-triangular shape proved to be more beneficial with superior properties[14]. In another opinion, the toxicity of AgNPs is regulated by the type of chemical and/or biological coatings on NPs surface[15], effecting the surface charges that define the cytotoxicity potentials. For instance, their positive surface charge renders them more suitable, more stable in the blood stream and postpones their clearance as compared to negatively charged NPs[16].

Synthesis

Techniques developed for synthesis of AgNPs are physical, chemical, and biological. Both physical and chemical methods are commercially more cost-effective, and the biological methods are relatively environmentally safe[17]. However, each method has its merits and its drawbacks.

1. Chemical methods

Equipment required for chemical production whether by heating or injection methods is more appropriate and simple than that used in biological methods. Evidently, Ag ions collect electrons from the reducing agent, convert into the metallic form, and then aggregate to form AgNPs. This method requires three modules: metal precursors, reducing agents, and stabilizing/capping agents. Basically, the reduction of Ag salts involves two stages: nucleation and subsequent growth[18].

In the chemical synthesis of AgNPs, silver nitrate ($AgNO_3$) is the common Ag salt precursor, with sodium borohydride and trisodium citrate as stabilizing factors. The former reducing agent is suitable for the manufacture of AgNPs with a size range of 5–20 nm, and the latter is appropriate for the fabrication of AgNPs with the size range 60–100 nm[19]. For production of AgNPs with less than 10 nm size, polyvinyl-pyrrolidone was found to act as size controller and capping agent with ethylene glycol as solvent and reducing agent[20]. Although very expensive, chemical approaches beneficially produce high yield. It was indicated that borohydride, 2-mercaptoethanol, citrate and thio-glycerol are hazardous complexes that produce toxic by-products during synthesis. Furthermore, it is extremely hard to manufacture AgNPs with a definite size, and it necessitates an additional step to avoid particle clustering[21].

2. Physical methods

Physically, AgNPs can be produced by evaporation–condensation or laser ablation systems. In the first process, it was noted that a maintained constant temperature of the heater surface generated polydispersed spherical NPs[22]. In the second system the laser wavelength markedly influences the particle size, where a decrease in wavelength trims down the average diameter of AgNPs[23]. Non-involvement of toxic reagents and employment of radiation as a reducing agent are the advantages of physical approaches of synthesis. However, long duration, solvent impurity, minimal yield, non-equivalent particle dispersion and

excessive energy consumption are the handicaps of such a type of synthesis[24].

3. Biological methods

Physical and chemical procedures for manufacture of AgNPs are expensive, time consuming and eco-unfriendly. This entails the development of environmentally friendly and cost-effectively approaches, to avoid chemical and physical complications of production. For application in health management, the biological methods comprise the use of fungi, bacteria, and yeasts as well as plant sources. It was recorded that NPs synthesis employing microorganisms and plants is safe, economic and is comparatively less deleterious to the environment than chemical synthesis. Moreover, microorganisms and plants absorb and assemble inorganic metallic ions from their adjoining environment[25,26].

The reduction of aqueous Ag ions using culture supernatants of various bacteria and fungi was proved to be a fast approach, generating AgNPs within five min only[25,27]. Biosynthesis using algae and yeast achieved NPs possessing high constancy and small size[28]. Employment of plant extracts "green synthesis" established a simple and quick approach for production of great AgNPs yields[29]. It was noted that deoxy-ribo-nucleic acid (DNA) can behave as a reducing agent, and that the high affinity of Ag ions with DNA N-7 phosphate and guanine base pairs makes DNA a template stabilizer[30].

Applications of AgNPs in parasitic diseases

I. Diagnostic applications

Plasmodium spp.

The World Health Organization (WHO) stated that the majority of commercially available malaria rapid diagnostic tests (RDTs) based on *Plasmodium* antibodies, are thermally affected in subtropical countries of the world. These tests functioned poorly at the proposed lower limit of detection (200 parasites/μl^2), when exposed to high storage temperatures. A histidine-targeted spectrophotometric sensor was advanced as a RDT, in which AgNPs were treated with nitrilotriacetic acid (NTA) for segregation and purification of histidine-tagged proteins. This sensor exhibited a respectable thermal stability and documented an augmented sensitivity in the detection of *P. falciparum* histidine rich protein (*Pf*HRP-II), which is an essential biomarker of malaria in serum samples[31].

Hemozoin, the by-product of *Plasmodium* parasite in erythrocytes was explored as a biomarker for early diagnosis of malaria. This led to laboratory preparation of β-hematin crystals that correspond to hemozoin biocrystals in spectroscopic characters. Additionally, magnetic NPs were chemically synthesized, formed of an iron oxide core and Ag shell. The surface-enhanced resonance Raman spectroscopy (SERRS) was supplemented with magnetic NPs and β-hematin crystals. The magnetic field boosted the attachment between crystals and magnetic NPs, affording five-time enhancement in SERRS signals. This technique certified a detection threshold of 5 nM β-hematin (equivalent to 30 parasites/ml), signifying its diagnostic potential for the early stages of malaria infection. Assessment of variable thicknesses of NPs revealed that the highest signal enhancement was accomplished using iron oxide NPs core of 40 nm thickness and AgNPs shell of 60 nm thickness[32,33].

In 2016, Chen and his colleagues[34,35] developed a procedure for recognizing hemozoin in the ring stage of *P. falciparum*. It was based on SERRS employment of AgNPs by two methods. In the first method, AgNPs are directly manufactured within parasites collected after the lysis of erythrocytes. In the second method, AgNPs are chemically produced separately and then mixed with parasites after erythrocyte lysis. The Raman spectra of hemozoin attained from parasites with *in vivo* directly-synthesized AgNPs, are compared to those from parasites mixed *in vitro* with separately-synthesized NPs. The outcome of both showed that interaction between hemozoin and AgNPs inside the parasite resulted in a weaker amplified Raman signal than parasites mixed with AgNPs. Therefore, the first method generated a greater sensitivity to a low parasitemia intensity, helping in the early diagnosis of malaria. While the second one showed a stronger correspondence between the anticipated hemozoin and the parasitemia intensity, which is favorable in estimation of the level of parasitemia. Moreover, this procedure provided a strategy to explore the mechanism of heme metabolism in *Plasmodium* and a tool to assess the effectiveness of anti-malaria agents[34,35].

Silver nanoclusters were proved to possess intense fluorescence emission and photo-stabilities. Therefore, a single strand oligonucleotide and a complementary oligonucleotide sequence were used as a template to synthesize the double-stranded DNA-scaffolded Ag nanoclusters (AgNCs-dsDNA). It was prepared to detect the important *P. falciparum* lactate dehydrogenase (*Pf*LDH) malaria biomarker. A significant luminescent enhancement was achieved with a low detection limit of 7.4 pg/μl, that is sensitive enough for clinical detection of *Pf*LDH in plasma from *Plasmodium*-infected patients. Moreover, a unique specificity of *P. falciparum* was demonstrated more than *P. vivax* and human lactate dehydrogenase. This was through a combined use of AgNCs-dsDNA with a selective single-stranded DNA aptamer against *Pf*LDH, providing a significant guarantee for malaria diagnosis[36].

Recently, adenosine monophosphate protected gold-silver bimetallic nanoclusters were used to develop a fluorescence probe for detection of *P. vivax* lactate dehydrogenase (*Pv*LDH). It recorded an extremely low limit of detection (3.7 ng/ml). The

observed high affinity was mainly attributed to the electrostatic interaction between the nanoclusters and *Pv*LDH, providing this probe with obvious high sensitivity and specificity[37].

Intestinal parasites

Recently, a label-free surface-enhanced Raman Spectroscopy (SERS) has been used for the detection of *C. parvum* oocysts along with AgNPs as SERS substrate. This technique was proved to be and efficient rapid tool to screen the water sources for such a parasite to guard against the occurrence of outbreaks[38].

II. Therapeutic applications

Toxoplasma gondii

Silver NPs were chemically prepared using trisodium citrate as a reducing agent. Their prophylactic and therapeutic prospects were evaluated in mice experimentally infected with *T. gondii* RH virulent strain. The preparations were orally administered in two concentrations: 100 µg/ml and 200 µg/ml, over a four-day regimen, either alone or in combination with polysaccharide (chitosan) NPs, with promising results. Silver concentrations were determined in different tissues (liver, intestine, kidneys, and lungs) of animals that received AgNPs. No silver was detected in the brain and all values of Ag detected in other tissues were within the safe range[39].

In 2017, a significant antiprotozoal activity of AgNPs of 10 nm size at a concentration of 10 µg/ml against tachyzoites of *T. gondii* RH 2F strain was proved. This was through growth inhibition and *in vitro* invasion assays, intracellular replication, and parasite infectivity assessment[40]. In addition, these AgNPs were conjugated with amino acid tryptophan and were investigated for their antiprotozoal effect on the tachyzoites of the same *Toxoplasma* strain at a concentration of 600 ng/ml[41]. These amino acid capped AgNPs suppressed parasite growth and invasion, decreased its viability and intracellular replication, and increased particles specificity for the parasites versus the host cells in a more potent way than uncapped AgNPs. Tryptophan-AgNPs revealed their host biocompatibility when checked for cytotoxicity in mammalian cells as human foreskin fibroblasts[41]. In another *in vitro* study, chemically synthesized AgNPs and their alloys with gold and platinum NPs were investigated for their activity against tachyzoites of *T. gondii* RH 2F strain with very promising impact on parasite viability[42].

The cystogenic *T. gondii* ME49 strain was used to assess the effect of chemically structured AgNPs used singly on parasite cyst formation through evaluation of *in vitro* differentiation by using an immunofluorescence assay. As a result, AgNPs obviously retarded tachyzoite to bradyzoite conversion and significantly reduced parasite cyst wall formation[43]. Furthermore, using a *Toxoplasma* strain expressing luciferase activity, PLK/DLUC_1C9, the effect of AgNPs at a concentration range of 0.01–200 µg/ml was tested on bradyzoite antigen 1 (BAG-1) promoter activity. These nanoparticles significantly decreased BAG-1 promoter activity under bradyzoite-inducing cell culture conditions relative to the untreated control, supporting their anti-bradyzoite potential[43].

Date palm seed (*Phoenix dactylifera*) extract and Nabka leaves (*Ziziphus spinachristi*) powder were tried in the green synthesis of AgNPs. Treatment with the resulting NPs showed anti-toxoplasmic activity with an obvious inhibition of hepatotoxicity and tissue inflammation in mice experimentally infected with *T. gondii* RH HXGPRT strain[44]. *Zingiber officinale* extract based AgNPs were synthesized and investigated *in vitro* at eight different concentrations (40, 20, 10, 5, 2.5, 1.25, 0.625, and 0.312 ppm) against RH strain tachyzoites, producing an evident effect on parasite viability. Notably, flow cytometry analysis revealed a high percentage of apoptotic tachyzoites after incubation with such NPs[45].

Biogenic AgNPs were synthesized through the reduction of AgNO3 with the fungal solution of *Fusarium oxysporum* strain. *In vitro* inoculation of cell culture with such NPs demonstrated reduction of *T. gondii* proliferation in the trophoblast cells and human chorionic villi with induction of the cellular inflammatory mediators[46]. In another *in vitro* experiment, these types of biosynthesized AgNPs showed no toxic effect on HeLa cell line at concentrations of 1.5-6 µM. Furthermore, these concentrations were tested on cells infected with *T. gondii* RH strain, revealing a statistically significant reduction in tachyzoite proliferation, cellular infection, and intracellular parasitic load. Such NPs were proved to induce autophagy and death of tachyzoites through apoptosis-like mechanism[47].

***Plasmodium* spp.**

Silver NPs were successfully synthesized from $AgNO_3$ through a green method using the leaves extract of *Andrographis paniculata Nees* as reducing as well as capping agent. Two concentrations (25 µg/ml and 100 µg/ml) of NPs tested on *P. falciparum* infected tissue culture, showed obvious inhibition of parasitemia[48]. Furthermore, the aqueous leaf extract of *Catharanthus roseus* (Linn) was efficient as a reducing agent in the biosynthesis of highly stable AgNPs. This herbal plant was proved to possess a high *in vitro* anti-plasmodial activity. Therefore, its extract exhibited a substantial enhancement of the antiparasitic effect of AgNPs at concentrations of 25, 50, 75, and 100 µg/ml on *P. falciparum* in cell cultures[49]. In a very interesting study, bioactive bacterial pigments were extracted from cultures of *Serratia marcescens* and *Chromobacterium violaceum*. Latex of *Jatropha gossypifolia* was successfully used in the green synthesis of AgNPs. The extracted microbial pigments prodigiosin and violacein were combined with phyto-synthesized AgNPs. An

obvious *in vitro* growth inhibition of chloroquine resistant *P. falciparum* FcB1/Colombia strain was obtained. Notably, pigment-AgNPs combinations recorded non-significant enhancement of cytotoxicity on peripheral blood mononuclear cells and human cancer cell lines; HeLa and MCF7[50].

The ethanol leaf extract of *Pteridium aquilinum L.* that belongs to Dennstaedtiaceae family, was efficiently utilized in biosynthesis of AgNPs as reducing and capping agent. Both leaf extract and bio-synthesized NPs revealed remarkable *in vitro* antiprotozoal efficacy on chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum*[51]. Additionally, *Azadirachta indica* seed extract, a member of Meliaceae, was employed as reducing and stabilizing agent for bio-fabrication of AgNPs. In the laboratory, these NPs showed a high anti-plasmodial activity against *P. falciparum* chloroquine-sensitive and resistant strains. Notably, through assessment of parasitemia, a moderate activity was reported in albino mice, infected with *P. berghei* after oral administration of biofabricated AgNPs in comparison to the reference drug 'Chloroquine'[52].

Another green approach for AgNPs synthesis, using aqueous leaf extracts of *Azadirachta indica* and *Ocimum sanctum* plants, was successfully employed. A culture of *P. falciparum* 3D7 strain which was maintained in human erythrocytes in RPMI 1640 medium was used to assess the anti-plasmodial activity of these green synthesized NPs with very promising results[53]. *Dicoma anomala* Sond. root extract was properly implemented in AgNPs biosynthesis. Bio-conjugation of the produced NPs with sesquiterpene molecule after its isolation from *Dicoma anomala* (Sond.) was also performed. Mouse fibroblast cell line was used to evaluate the antiplasmodial activity of biogenic AgNPs and AgNPs-conjugated sesquiterpene against *P. falciparum* NF54 strain. Notably, it was proved that the antiplasmodial potency of such a compound (sesquiterpene) was increased by loading on AgNPs, synthesized by the same plant root[54].

In a recent study, green AgNPs were prepared from the leaves extract of two Artemisia species (*A. abrotanum* and *A. arborescens*). The hemocompatibility and the antimalarial efficiency were studied on cultures of *P. falciparum* PAPA, FcB1, It-G and ARS1 laboratory strains, exploiting rising doses of these NPs (0.6 to 7.5 µg/ml) on parasitized red blood cells (pRBCs). The outcomes exhibited that the AgNPs hemo-compatibility is linked to their synthetic approach and the implemented dose. *A. abrotanum*-based AgNPs documented the lowermost percentage of hemolytic activity on pRBCs, emphasizing their hemo-compatibility. The anti-malarial activity of *A. abrotanum*-based AgNPs was higher than that of A. arborescens-based ones. Furthermore, *in vitro* treatment with *A. abrotanum*-based AgNPs stopped the parasite growth in the ring stage, proving their proficiency to hinder the development of *P. falciparum*[55]. In another experiment, *Salvia officinalis* leaf extract was employed to produce AgNPs biologically. Interestingly, the antimalarial activity of such NPs was checked in a murine model of *P. chabaudi* at an oral dose of 50 mg/kg for one week and two weeks. The outcomes revealed that both pre-treatment (treatment before experimental infection) and post-treatment (treatment after experimental infection) elicited a substantial decline in parasitemia, relative to the infected untreated group. In addition, the hepatoprotective consequences of *S. officinalis* leaf extract-biosynthesized AgNPs were evidenced by diminution in the *P. chabaudi*-induced inflammatory and hepatic oxidative stress indicators[56].

Leishmania spp.

Solutions of AgNPs at concentrations of 1.6, 3.2, 6, 25, 12.5, 25, 50 and 100 ppm reduced proliferation of amastigotes of *L. major* MRHO/IR/75/ER strain in tissue culture. However, it was not a statistically significant reduction when compared with the control wells. In addition, nanosilver solutions could not significantly decrease the lesion sizes and amastigote counts in mice, when administered topically at doses of 100 ppm, 500 ppm and 1000 ppm on the local lesions daily for 14 days. Interestingly, AgNPs seemed to be effective for control of secondary infection of such localized cutaneous leishmaniasis lesions[57].

Allahverdiyev *et al.*[58] investigated the *in vitro* effects of chemically synthesized AgNPs on biological characteristics of *L. tropica* MHOM/TR/99/EP39 strain, involving parasite morphology, metabolic activity, proliferation, infectivity, and viability. The effects of different concentrations, of AgNPs (25, 50, 100, 150, and 200 µg/ml) were studied under dark and under ultraviolet (UV) light exposures. In the dark AgNPs disturbed the morphology, and infectivity, inhibitd propagation and metabolic activity of promastigotes, and the survival of amastigotes in J774 macrophage cells. Of note, the anti-leishmanial outcomes were significantly boosted in the presence of UV light[58].

The AgNPs concentration of 0.2-4 µM proved leishmanicidal for amastigotes of *L. infantum* in murine macrophages. Using AgNPs successfully encapsulated by ferritin molecules *in vitro* on both promastigote and amastigote stages, showed that ferritin-AgNPs increased the death of both stages at micro molar concentrations[59]. In another study, AgNPs were prepared by a dual procedure: chemical (Chem-AgNPs) and biological (from the fungus *Fusarium oxysporum*) (Bio-AgNPs), at variable concentrations. When tested *in vitro* against promastigotes of *L. Amazonensis* (WHOM/BR/75/Josefa) transfected with the gene of green fluorescent protein, Bio-AgNPs were four-fold more potent than chemically formulated ones[60]. *In*

vivo, they were assessed in the treatment of cutaneous leishmaniasis in mice infected in the ear, using amphotericin B as reference drug. Mice were treated twice a week with intralesional injections of 6.5 µg/Kg, 21.6 µg/Kg of Bio-AgNPs and Chem-AgNPs, respectively for four weeks. Bio-AgNPs revealed an efficacy equivalent to 300-fold higher doses of amphotericin B. They were more effective than three-fold higher doses of Chem-AgNPs. It is worth noting that the *in vivo* AgNPs toxicity was assessed 42 d post infection, through estimation of serum aspartate transaminase, alanine transaminase and creatinine. Contrary to the hepato- and nephrotoxicity which was provoked by amphotericin B treatment, a negligible hepatotoxicity was generated by Chem-AgNPs and no toxicity was noticed with Bio-AgNPs. These data indicated that the biogenic formulation resulted in enhanced *in vitro* and *in vivo* anti-leishmanial performances, and less harmful AgNPs as compared to the chemical formulation[60].

Titanium dioxide (TiO_2)-Ag nanocomposite was chemically prepared for assessment of its anti-leishmanial action on *L. tropica* (MHOM/TR/99/EP39) and *L. infantum* (MCAN/TR/2005/EP126) promastigotes. This nanocomposite had prominently disturbed the parasite biological proficiency such as survival and metabolic activity, both in the dark and under visible light. The leishmanicidal pursuit was also perceived against amastigotes cultured on J774 macrophage cell line[61]. Various dilutions of Chem-AgNPs (200, 20, and 2 µg/ml), gold NPs, TiO_2 NPs, zinc oxide NPs, and magnesium oxide NPs were trialed *in vitro* on *L. major* promastigotes. Multiple biological parameters such as parasite viability, proliferation, and infection index were judged under UV rays, infrared (IR) rays, and dark settings after 24-h exposure to NPs. The highest anti-leishmanial performance was recorded for AgNPs; and both UV and IR light augmented the antiparasitic activity in all tested NPs[62].

Various concentrations of Chem-AgNPs significantly decreased the count of viable *L. major* promastigotes *in vitro* however, the particles did not completely kill promastigotes. Notably, the combined use of both AgNPs and direct current electricity of 3 mA for ten min showed a significant synergistic effect on parasite mortality[63]. The healing effect of AgNPs in cutaneous lesions caused by *L. major* in mice was proved by intralesional injection on a daily basis for four weeks. However, glucantime, the standard therapy showed more potent healing activity[64]. An electric method was successfully employed in synthesis of AgNPs. These NPs were administered intralesional in BALB/c mice subcutaneously infected with *L. major*. In another infected-mice group, the cutaneous lesions were subjected to phototherapy of ultraviolet B (UVB) while the third group received both AgNPs and UVB for four sessions. The highest parasite inhibitory effect was observed in the group receiving AgNPs plus UVB. Interestingly, determination of splenic parasite-burden proved the ability of such type of irradiation in the presence of AgNPs, to inhibit the spread of cutaneous lesions and reduce the rate of visceral progression of the infection[65]. Green synthesis of Ag and TiO_2 NPs was performed using the aqueous leaf extract of *Euphorbia prostrata* (Euphorbiaceae). Both metallic NPs were tested separately on *L. donovani* (strain MHOM/IN/80/DD8). Bio-fabricated AgNPs were the most active against both parasitic stages after 24-h *in vitro* exposure, with 50% inhibitory concentrations of about 15 µg/ml and 4 µg/ml in promastigotes and intracellular amastigotes, respectively[66]. Later, the hydrothermal method was applied for synthesis of TiO_2-AgNPs and investigated *in vitro* for anti-leishmanial properties, either alone or in combination with *Nigella sativa* oil. This combination demonstrated obvious killing effects on *L. tropica* promastigotes and amastigotes with inhibition of their metabolic activities and reduction of infection index of macrophage cell line[67].

Chitosan-based AgNPs were synthesized from $AgNO_3$, using sodium borohydride as a reducing agent, and a polysaccharide biopolymer "chitosan" as a capping agent. Investigated using 0.42 to 27 µg/ml concentrations they were found to induce noticeable *in vitro* activity against promastigotes of *L. amazonensis* (IFLA/BR/67/PH8) strain with intermediary cytotoxic consequence on murine macrophages. In addition, chitosan based AgNPs obviously declined the count of internalized amastigotes in infected macrophages. Notably, their excellent anti-leishmanial activity seems to be related to a synergistic effect between the AgNPs and chitosan solution used in NPs stabilization[68]. Silver NPs were also phyto-fabricated using *Sechium elude* fruit extract, which served as both reducing and capping agent. The anti-leishmanial potential of such NPs was judged on the clinical *L. donovani* promastigotes isolates *in vitro*, resulting in IC50 value of about 52 µg/ml. Moreover, when added to normal mammalian monocyte cell line a non-statistically significant toxicity was produced[69].

Silver oxide NPs (AgO_2NPs) were successfully prepared, using *Ficus benghalensis* prop root extract as a reducing agent. The green biosynthesized NPs were investigated *in vitro* at different concentrations (25, 50, 100, 200, and 300 µg/ml) on *L. donovani* (DUAA/IQ/2005/MRU15) promastigotes. The results indicated their marked inhibitory effects on the parasite growth when compared to both the standard anti-leishmanial drug (Pentostam) and the untreated control group[70].

Biogenic gold-silver bimetallic NPs (Au-Ag NPs) were synthesized through a single-step reduction process using aqueous leaf extracts of three plants: fenugreek, coriander, and soybean. Different concentrations of such NPs (0.01, 0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 1.25, and 2.5 µg/ml) were tested

in vitro against *L. donovani* (ATCC/Dd8). These NPs induced apoptosis-like death in the promastigotes with marked decrease in intracellular amastigote-burden after 48 h exposure[71]. Silver NPs were biosynthesized using aqueous extracts of *Ficus carica* fruits and *Olea europaea* leaves. Theses NPs were proved to possess both protective and therapeutic effects on *L. major*-induced cutaneous leishmaniasis in BALB/c mice. This was through obvious reductions in the average size of cutaneous lesions when compared to the untreated mice. Biochemical, histopathological, and molecular analyses showed the high anti-inflammatory and antioxidant impacts of such NPs leading to a faster clinical efficacy than standard Pentostam treatment[72].

The aqueous rhizome-extract of ginger was efficient in the biosynthesis of AgNPs. These NPs were proved to exhibit a remarkable *in vitro* leishmanicidal effect on *L. major* promastigotes and intracellular amastigotes in a concentration dependent manner. Parasitic cell apoptosis and necrosis were demonstrated after 72-h exposure to biosynthesized AgNPs[73].

Commiphora molmol (myrrh) is a plant whose aqueous extract has been recently applied in the green fabrication of AgNPs. The antiparasitic pursuit of the Chem-AgNPs and myrrh-based AgNPs was assessed on *L. major* both *in vitro* and *in vivo*[74]. Five concentrations of NPs (10, 50, 80, 100, and 150 µL/100 µL) were tested on *L. major* promastigotes *in vitro*. All concentrations presented a substantial promastigote propagation inhibition. Of note, the higher concentrations of myrrh-based AgNPs (100, 150 µL/100 µL) recorded a significantly greater *in vitro* inhibitory effect on the parasite growth in comparison to the Chem-AgNPs and the standard drug "Pentostam" at the same concentrations. Myrrh-based AgNPs were also applied topically, once daily, on cutaneous lesions in experimentally infected mice until the lesions healed entirely. Interestingly, complete healing of the lesions was achieved by the treatment with Myrrh-based AgNPs in 21 d, while Chem-AgNPs and Pentostam exhibited a moderate healing effect on the lesions. Thus, the anti-leishmanial activity of green synthesized AgNPs was more powerful than Chem-AgNPs and Pentostam both in the *in vitro* and *in vivo* investigations. Furthermore, there were negligible changes in serum creatinine levels in AgNPs-treated animal groups. Accordingly, AgNPs had expressively lowered the liver function serum markers, suggesting the safety of such nano formulas[74].

Trypanosoma spp.

In an *in vitro* experiment, three different species: *T. congolense* IL3000 (a savannah-type strain), *T. evansi* (Tansui) and *T. brucei brucei* (GUT) at 3.1, were subjected to various intensities (1 to 10 µg/ml) of chemically synthesized, tannic acid-stabilized AgNPs[42]. Parasite growth curves were established by quantifying the ATP concentrations of the viable parasites, using a luminescence-based cell viability assay. AgNPs exploited a robust and selective activity against *T. brucei brucei* but moderate activity against *T. congolense* and *T. evansi*. Furthermore, when tryptophan conjugated AgNPs were investigated for anti-trypanosomal activity, they appreciably suppressed the growth of *T. brucei brucei* by 90% but had inconsiderable effect on *T. congolense* or *T. evansi* (20 and 40% parasite propagation inhibition, respectively). Contrary, gold and platinum alloys with AgNPs failed to overpower the growth of the three *Trypanosoma* spp., proposing that combining the individual NPs as alloys reduces their individual anti-parasitic potentials. It is noteworthy, the calculation of the ratio of the mammalian cytotoxicity to the anti-parasite efficacy revealed a favorable development in which AgNPs showed ≥200-fold selectivity toward the various parasite species versus the mammalian cells, signifying their specificity for *Trypanosoma* elimination. *In vivo* evaluation of such NPs was implemented using Wistar rats, experimentally overwhelmed with *T. brucei brucei* (Lafia strain). Animals were treated with AgNPs at two doses, 5 µg/kg and 10 µg/kg. Unfortunately, there was no remarkable decline in the parasite burden in AgNPs-treated animals when compared with the untreated control. The NPs appeared to possess only a trypanostatic effect and failed to appreciably lengthen the survival duration of infected rats[42].

Recently, xylan, a bioactive polysaccharide, extracted from corncob, has been efficiently employed as a bioreductor in AgNPs green synthesis. These NPs were tested for the *in vitro* anti-parasitic potential at concentrations of 0.25 mg/ml, 1 mg/ml and 10 mg/ml on the epimastigote forms of *T. cruzi* Y strain. The mechanisms of parasitic cell death through apoptosis/necrosis were investigated by parasite labelling with Annexin/Propidium iodide and analysis by flow cytometry. Xylan-based AgNPs revealed a considerable increase in the percentage of cell death by necrosis (98%) at the concentration of 100 µg/ml[75].

Acanthamoeba castellanii

Aqueous leaf-extracts of *Jatropha curcas, Jatropha gossypifolia* and *Euphorbia milii* plants were utilized for AgNPs phytosynthesis. These plant extracts were tested for the amoebicidal activity and showed a little *in vitro* effect on the viability of *A. castellanii* trophozoites. However, phytosynthesized AgNPs at concentrations of 50, 25, 12.5, 6.25, 3.125 and 1.56 µg/ml revealed a higher amoebicidal activity even at the least concentration (1.56 µg/ml)[76]. In another study, amphotericin B, nystatin, and fluconazole were conjugated with chemically fabricated AgNPs. The conjugated and unconjugated drugs were investigated against *A. castellanii*. The parasite viability assays revealed that AgNPs conjugated with amphotericin B and nystatin exhibited significant amoebicidal

properties when compared with AgNPs alone or the drugs alone. However, fluconazole conjugation with AgNPs had minimal effect on its antiamoebic activity. These results could be taken in consideration for development of effective vehicles for drug delivery[77].

Tannins are polyphenolic plant metabolites. Tannic acid modified AgNPs and unmodified AgNPs were tested against clinical strains of *Acanthamoeba* spp. and *A. castellanii in vitro*. The modified AgNPs showed a significant anti-amoebic effect moreover, they were well absorbed by the trophozoites and did not induce encystation. On the other hand, unmodified AgNPs recorded a minimal anti-amoebic activity[78]. Three well-known contact lens disinfecting solutions were conjugated with AgNPs at concentrations of 0.25, 0.5, 1.25 and 2.5 ppm to be assessed *in vitro* for their anti-amoebic activity against *Acanthamoeba* trophozoites. In addition, cytotoxicity assays were performed on the fibroblast HS-5 cell line. The anti-amoebic efficacy of contact lens solutions was significantly increased by conjugation with such NPs in a dose dependent manner with a low cytotoxicity profile. This study provided a promising approach for prevention of *Acanthamoeba* keratitis among contact lens users[79].

Green-synthesized AgNPs were prepared, using the aqueous solution of gum acacia. These NPs were loaded with the flavonoids of citrus fruits, hesperidin and naringin and studied as antimicrobial agents against *A. castellanii* trophozoites. The *in vitro* amoebicidal assays revealed that hesperidin loaded AgNPs had quantitatively abolished parasite viability by 100% with inhibition of its encystation and excystation by 85%. Furthermore, they completely aborted parasite-mediated host cell pathogenicity[80]. Another *in vitro* study was designed to investigate the anti-acanthamoebic effects of naturally occurring unsaturated fatty acid (Oleic acid), both alone and in conjugation with chemically manufactured AgNPs. Viability, growth inhibition, encystation, excystation, and parasite-mediated host cell cytotoxicity assays revealed that both compounds exhibited significant anti-amoebic properties. Further enhancement of the efficacy of oleic acid was achieved by conjugation with AgNPs[81].

Tannic acid-modified AgNPs had enhanced the anti-*Acanthamoeba* activities of three contact lens-care solutions without increasing their cytotoxicity profiles on human fibroblast HS-5 cell line[82]. Synthetic tetrazoles and tetrazoles-conjugated AgNPs were subjected to anti-*Acanthamoeba in vitro* studies. Viability, encystation and excystation assays discovered promising anti-amoebic performances which were more apparent in tetrazole-conjugated AgNPs. This was attributed to the amplified bioavailability of AgNPs that permit better transport of therapeutic compounds to parasites. Furthermore, these NPs had significantly trimmed down cytopathogenicity of *A. castellanii* with minimal cytotoxicity on HeLa cell line[83]. Additionally, in the same year, the same investigators demonstrated the remarkable *in vitro* power of metformin-coated AgNPs to disturb the viability and inhibit the propagation of *A. castellanii* more than metformin and AgNPs lonely. Metformin-coated AgNPs also blocked parasite encystation and hindered its excystation after 72-h incubation. This study was considered an advance in drug delivery proficiency of such NPs[84].

Naegleria fowleri

Oleic acid and its conjugate with AgNPs were prepared for investigation of their *in vitro* antiamoebic effects against *N. fowleri*, in comparison to the standard therapeutic drug (amphotericin B). Parasite viability and host cell cytopathogenicity assays were applied. Interestingly, the nano-conjugate of oleic acid exhibited significantly higher amoebicidal effects than oleic acid alone and amphotericin B with minimal toxic effect on HeLa cell line[85]. The aqueous solution of gum acacia was used in the green synthesis of AgNPs. These NPs were loaded with hesperidin and naringin flavonoids to check their antimicrobial activities against *N. fowleri* trophozoites. The *in vitro* amoebicidal assays revealed that hesperidin-loaded AgNPs had markedly abolished parasite viability with obvious inhibition of parasite-mediated host cell pathogenicity[80].

Entamoeba histolytica

Silver NPs were chemically fabricated using a stabilizing agent, sodium dodecyl sulphate, and two reducing agents' hydrazine hydrate and sodium citrate. Four concentrations (0.5, 1, 2 and 4 mg/L) were prepared to investigate the *in vitro* anti-amoebic potential of AgNPs regarding *E. histolytica* cysts. There was a statistically significant high cyst mortality rate after 3-h exposure at a concentration of 4 mg/L[86]. Recently, a highly biocompatible protein-coated AgNPs formulation exhibited a remarkable amoebicidal effect on *E. histolytica* trophozoites cultured in TYI-S33 medium after 24-h exposure[87].

Giardia lamblia

In an *in vivo* study, Chem-AgNPs were evaluated as oral treatment for rats experimentally infected with *G. lamblia* by determining parasite burdens in stool samples and intestinal tissues. The best monotherapy effect was detected in AgNPs-treated animals and the highest combination-therapy yield was demonstrated in animals treated by AgNPs combined with either chitosan or curcumin NPs. Notably, the accumulated Ag in different organs was within the safe limits[88].

***Cryptosporidium* spp.**

The *in vitro* antiprotozoal activity of chemically structured AgNPs was assessed on *C. parvum* oocysts. The recorded mortality rates were 47% and 73% after 3-h exposure at concentrations of 0.5 mg/L and 1 mg/L

respectively. Of note, AgNPs concentrations of 2 mg/L and 4 mg/L induced 100% oocyst mortality[86]. *In vitro* exposure of *C. parvum* oocysts to proteinate capped AgNPs resulted in obvious oocyst morphological alterations and markedly decreased the excystation rates of sporozoites. Moreover, ceramic water filters impregnated with capped AgNPs enhanced water disinfection against such oocysts. Using a murine model, the treatment of oocyst infective-inoculum with proteinate-capped AgNPs for 30 min, significantly decreased the animal infection rate[89].

Cyclospora cayetanensis

Chemically synthesized AgNPs were investigated for their anti-*Cyclospora* effects in both immunocompetent and immunosuppressed experimentally infected mice in comparison to the standard therapy (trimethoprim-sulfamethoxazole combination). The effect was determined through assessment of stool oocyst-load, viability, and ultrastructural changes. Toxicity profile was also demonstrated by measurement of hepatic and renal enzymes in sera. Results revealed that AgNPs elicited a statistically significant reduction in oocyst burden and viable oocysts count in stool[90].

***Blastocystis* spp.**

Younis *et al*.[91] conducted a study to evaluate the efficacy of chemically synthesized AgNps alone and in combination with metronidazole as potential alternative therapeutic agents for *B. hominis*. They reported statistically significant differences in the *in vitro* growth inhibition of *B. hominis* in a time dependent way, using AgNPs in a concentration of 150 µg/ml. The maximum anti-parasitic effect was determined for AgNPs combined with metronidazole after three-h exposure[91].

***Echinococcus granulosus* (hydatid cyst)**

A biogenic AgNPs, derived from the aqueous fungal extract of *Penicillium aculeatum* was evaluated *in vitro* against protoscoleces of hydatid cysts. Different NPs concentrations (0.025, 0.05, 0.1, 0.15 mg/ml) were tested at different exposure times (10 to 30, 60, 120 min). At 0.1 and 0.15 mg/ml dilutions, the mortality rates recorded 83% and 90% after 120 min exposure respectively[92]. Recently, in addition to AgNPs another *in vitro* scolicidal assessment of chemically manufactured, iron, copper, silica, and zinc oxide NPs was attempted against hydatid cyst protoscoleces. Concentrations of 0.25, 0.5, and 1 mg/ml were attempted for 10, 30 and 60-min incubation. Results revealed that all tested concentrations of AgNPs, had remarkable scolicidal effects compared to other NPs[93].

***Fasciola* spp.**

A biomass of *Trichoderma harzianum* fungus was employed in the synthesis of AgNPs. In an *in vitro F. hepatica* egg hatching experiment, the hatching rate was 100% for non-treated control eggs and about 29% for triclabendazole-treated eggs. Interestingly, the egg hatching rate was markedly decreased to about 9% after 14 d exposure to triclabendazole combined with biologically synthesized AgNPs at a concentration of 50 µg/ml[94].

Trichinella spiralis

The influence of chemically and biosynthesized AgNPs was investigated on the *in vitro* viability and infectivity of *T. spiralis* muscle larvae. The used AgNPs were chemically prepared using sodium borohydride as reducing agent and polyvinyl-pyrrolidone as a stabilizer. On the other hand, biosynthesized AgNPs were prepared using the methanolic extract of *Commiphora myrrha* plant. A 100% mortality rate was observed after 48-h exposure to either chemically or biosynthesized AgNPs at concentrations of 10, 15 and 20 µg/ml. Moreover, this high mortality rate was also achieved by biosynthesized NPs at a concentration of 5 µg/ml. Additionally, a complete inhibition of the larval infectivity was reported after 24 h exposure to sub lethal doses of chemical and myrrh prepared AgNPs[95].

Brugia malayi

An *in vitro* study demonstrated the microfilaricidal activity of Chem-AgNPs. The NPs induced a marked reduction in the motility of *B. malayi* microfilariae and an evident decrease in parasite viability. The ultrastructural study and the differential staining assays revealed apoptotic death of parasites under the effect of such NPs, thus supporting their potential as a drug candidate against lymphatic filariasis[96].

III. Protective applications for prevention of disease transmission

Insecticidal activities

Dangerous human infections transmitted by mosquitoes, result in millions of annual deaths. Synthetic insecticides for the control of vector mosquitoes, provoked physiological resistance and unfavorable environmental effects in addition to elevated operational expenses. Hence, nanotechnology presented a research field with various applications in vector control programs. Silver NPs synthesized using natural products for vector control have been the main aim in this field.

Biosynthesized AgNPs with aqueous leaf extract of *Eclipta prostrata*, a member of the Asteraceae, presented a reducing and shape-directing agent. It was investigated as larvicidal for fourth instar larvae of *C. quinquefasciatus*, a filariasis vector, and *An. subpictus*, a malaria vector. This NP biological product possessed the potential of an ideal ecofriendly method for the control of these vectors[97]. Another aqueous leaf extract from *Musa paradisiaca* (Musaceae) was effectively employed for reducing and capping material in green synthesis of AgNPs. Various concentrations of these NPs (5.0, 4.0, 3.0, 2.0 and 1.0 mg/L) were investigated against the fourth instar larvae of *An. stephensi*, one of malaria vectors, achieving high mortality rates[98].

The aqueous extract from green leaves, dry leaves, and green berries of *Solanum nigrum L.* (Solanaceae) were applied in AgNPs-biosynthesis. Nanoparticle concentrations of 2.5, 5, 10 ppm were tested on the second and third instar larvae of *An. stephensi* and *C. quinquefasciatus*. The larvicidal bioassay revealed the highest mortality at 10 ppm of dry leaf-synthesized-AgNPs against *An. stephensi*[99]. Silver NPs were synthesized using the aqueous leaf extract of *Vinca rosea* (Apocynaceae) as both reducing and capping material. The antiparasitic effects of varying concentrations of the extract and biosynthesized AgNPs were investigated against the fourth instar larvae of *An. stephensi* (Liston) and *C. quinquefasciatus* (Say). The maximum efficacy was recorded for NPs with LC50 of 12 and 17 mg/ml after 48 and 72 h exposure respectively[100]. Additionally, the aqueous leaf extract of another member of Apocynaceae (*Nerium oleander*) and floral extract of *Chrysanthemum indicum* (Asteraceae) were used as reducing and capping agents for plant-mediated AgNPs-synthesis. Notably, the insecticidal activity of such NPs was recorded against the first to fourth instar larvae and pupae of *An. stephensi*[101,102]. Furthermore, *Arachis hypogaea* aqueous peel-extract was properly used in AgNPs bio-synthesis. The larvicidal activity of such NPs was tested against the fourth instar larvae of *Ae. aegypti* and *An. stephensi*. The larvae of the two mosquito species were highly susceptible to AgNPs; 100% mortality was documented at 15 mg/L concentration, after 24-h exposure[103]. Bio-fabricated AgNPs using *Annona reticulata* leaf aqueous extract were applied to the fourth instar larvae of *Ae. aegypti* at different concentrations (3-20 µg/ml). A 100% mortality was recorded at AgNPs concentration of 20 µg/ml after 24-h exposure with LC50 of 4.43 µg/ml[104].

Green synthesis of AgNPs was implemented using the aqueous leaf-extracts of *Feronia elephantum* (Rutaceae), *Sida acuta* (Malvaceae), *Chomelia asiatica* (Rubiaceae) and *Annona muricata* (Annonaceae). The AgNPs products were investigated against the third instar larvae of *An. stephensi*, *Ae. aegypti*, and *C. quinquefasciatus*. Significant mortality was reported after 24 h exposure to plant extracts for all three mosquitoes' larvae. Green-synthesized AgNPs recorded a greater detrimental effect at concentrations of 5-25 µg/ml, 10-50 µg/ml, 8-40 µg/ml, 6-30 µg/ml against the larvae of all three vectors, respectively[105-108]. In addition, the larvicidal potency of methanol, hexane, acetone, chloroform, and aqueous bark extracts of *Holarrhena antidysenterica* (Apocynaceae) and AgNPs synthesized through the usage of the aqueous bark-extract was attested against the third instar larvae of *Ae. aegypti* and *C. quinquefasciatus*. These NPs recorded an extremely higher larval mortality than the various plant bark-extracts even at low doses after 72-h exposure[109]. Furthermore, the aqueous leaf extract of *Piper longum*, a member of the family Piperaceae was successfully employed in the fabrication of AgNPs. Methanol, ethyl acetate, chloroform, hexane, and aqueous leaf extracts were also formulated separately. The various leaf extracts and AgNPs were reviewed for their bio-efficacies against the third instar larvae of *An. stephensi*, *Ae. aegypti*, and *C. quinquefasciatus* resulting in obvious mortality rates after 72-h exposure[110].

The aqueous leaf extracts of two members of Lamiaceae; *Anisomeles indica Kuntze* and *Clerodendrum chinense* were utilized efficiently in bio-reduction of Ag nitrate into AgNPs. Both leaf extracts and bio-synthesized AgNPs exerted larvicidal activities against the third instar larvae of the malaria vector "*An. subpictus*", the dengue fever vector "*Ae. albopictus*" and the Japanese encephalitis vector "*C. tritaeniorhynchus*". Silver NPs were tried at 15, 30, 45, 60, and 75 mg/ml concentrations for 24 h, giving statistically significant higher larval mortality rates in all tested species[111, 112].

Another aqueous leaf extract of *Datura metel* (Solanaceae) proved insecticidal against the first instar larvae and pupae of *An. stephensi*. Notably, this plant extract was used as a reducing and stabilizing agent for the biosynthesis of AgNPs, and demonstrated obvious *An. stephensi* larvicidal and pupicidal properties at eco-friendly low quantities. Interestingly, such NPs disturbed the predacious efficiency of the odonates aquatic predators that trap mosquito larvae. Under standard laboratory circumstances after 24 h exposure, the predation efficiency of dragonfly nymphs, *Anax immaculifrons*, was around 75% and 53% for the second instar and third instar larvae, respectively. Biosynthesized AgNPs in the aquatic ecosystem intensified the predation rates to 95% and 78%, respectively[113]. The implementation of the aqueous leaf extract of *Mimusops elengi* as a reducing and stabilizing agent proved proficient in the manufacture of AgNPs. Under laboratory settings, minimal quantities of such NPs displayed first instar larvicidal, pupicidal and adulticidal properties on the malaria vector (*An. stephensi*) and the arbovirus vector (*Ae. albopictus*)[114]. Field settings application of *Mimusops elengi* extract and biosynthesized AgNPs yielded 100% larval death after 72 h for both mosquito species. All in all, the predation efficiency of the mosquito larvivorous fish, *Gambusia affinis*, for the third instar larvae of *An. stephensi* and *Ae. albopictus* was about 86% and 81%, respectively. However, in AgNPs mixed environments, the predation was raised up to 94% and 89%, respectively. This experiment documented that *Mimusops elengi*-synthesized AgNPs could be employed at ultra-low quantities to downgrade larval populations of such vectors, without unfavorable effects on predation rates of mosquito natural enemies[114].

The insecticidal activity of AgNPs, synthesized using *Feronia elephantum* aqueous leaf extract was determined against the adults of *An. stephensi*, *Ae. aegypti*, and *C. quinquefasciatus*. There was an obviously high mortality after the exposure to *Feronia elephantum* extract for all three mosquito species.

However, higher mortality rates were recorded by green synthesized AgNPs for all tested species[106]. In a comprehensive study, AgNPs were bio-synthesized using the ethanol leaf extract of *Pteridium aquilinum* (*L.*) a member of Dennstaedtiaceae family. These NPs showed obvious multi-stage insecticidal activities against the first to fourth instar larvae and pupae of *An. stephensi* in the laboratory conditions at different concentrations (3.125, 6.25, 12.5, 25, and 50 ppm). Furthermore, in the field application, bio-synthesized AgNPs resulted in 100% mosquito larval reduction after 72 h and significantly reduced the longevity and fecundity of laboratory reared *An. stephensi* adults[51]. In another experiment, *Azadirachta indica* seed extract, a member of Meliaceae, was tried in bio-fabrication of AgNPs as reducing and stabilizing agent. The viability of laboratory-reared pupae as well as the first, second, third and fourth instar larvae of *An. stephensi*, was substantially affected after 24 h exposure to such NPs[52].

Notably, the insecticidal potency of AgNPs is not restricted to mosquitoes only but also extended to ticks. Silver NPs were fabricated using *Ocimum canum Sims* (Lamiaceae) aqueous leaf extract. Such NPs had elicited a remarkable acaricidal activity against the larvae of an Ixodide tick "*Hyalomma marginatum isaaci*", which is responsible for transmission of Crimean–Congo haemorrhagic fever virus to humans[115].

Molluscicidal activities

An important experiment was proposed to evaluate the *in vitro* effects of chemically synthesized AgNPs on *S. mansoni* cercariae and its intermediate host; *B. alexandrina* snails as well as assessment of their effects on the cercarial infectivity *in vivo*. This study evidenced that AgNPs retain a remarkable *in vitro* molluscicidal activity, causing 100% mortality at a concentration of 30 µg/ml with an obvious hindrance of snail-infection with *S. mansoni* miracidia at a concentration of about 10 µg/ml. Moreover, AgNPs at 50 µg/ml had significantly amplified the mortality of the cercariae in a dose- and time-dependent mode, up to 100% mortality after one-h exposure. *In vivo*, parasitological parameters made known that AgNPs had prohibited the animal infection when cercariae were treated before experimental infection, either through the tail immersion or subcutaneous route. This reinforced the marked diminution of cercarial infectivity under the effect of such NPs. It is worth noting that AgNPs could play a valuable function in prevention of schistosomiasis transmission[116].

In summary, all studies discussed in the present review are listed in tables (1-4). Briefly, the plants implemented in the green synthesis of AgNPs to assess their anti-parasitic potentials and insecticidal activities were listed in tables (1 and 2), respectively. Furthermore, the parasites affected by AgNPs were gathered in table (3), while arthropods disrupted by such NPs were listed in table (4).

CONCLUDING REMARKS

1. Biosynthesized and green synthesized AgNPs are ecofriendly efficient alternatives of chemically synthesized NPs.

Table 1. List of plants implemented in the green synthesis of AgNPs to assess their anti-parasitic potentials.

Scientific name	Plant part	Extract	Parasite	References
Phoenix dactylifera	Seeds	Aqueous	***T. gondii***	[44]
Ziziphus spina-christi	Leaves	Methanol		
Zingiber officinale	NI	NI		[45]
Andrographis paniculata	Leaves	Aqueous	***Plasmodium* spp.**	[48]
Catharanthus roseus				[49]
Pteridium aquilinum		Ethanol		[51]
Azadirachta indica	Seeds	Aqueous		[52]
	Leaves			
Ocimum sanctum	Leaves			[53]
Dicoma anomala	Roots			[54]
Artemisia abrotanum ***Artemisia arborescens***	Leaves	Ethanol		[55]
Salvia officinalis	Leaves	Aqueous		[56]
Sechium elude	Fruits	Aqueous	***Leishmania* spp.**	[69]
Ficus benghalensis	Roots			[70]
Commiphora molmol	Oleo-gum resin			[74]
	Oleo-gum resin	Methanol	***T. spiralis***	[95]

NI: Non-identified.

Table 2. List of plants implemented in the green synthesis of AgNPs to assess their insecticidal activities.

Scientific name	Plant part	Extract	Parasite	References
Pteridium aquilinum	Leaves	Ethanol	***Anopheles* spp.**	[51]
Azadirachta indica	Seeds	Aqueous		[52]
Nerium oleander	Leaves	Aqueous		[101]
Chrysanthemum indicum	Flowers	Aqueous		[102]
Datura metel	Leaves	Aqueous		[113]
Musa paradisiaca	Leaves	Aqueous		[98]
Eclipta prostrata	Leaves, berries	Aqueous	***Anopheles, Culex* spp.**	[97]
Solanum nigrum	Leaves	Aqueous		[99]
Vinca rosea	Leaves	Aqueous		[100]
Arachis hypogaea	Peel	Aqueous	***Anopheles, Aedes* spp.**	[103]
Mimusops elengi	Leaves	Aqueous		[114]
Annona reticulate	Leaves	Aqueous	***Aedes* spp.**	[104]
Sida acuta	Leaves	Aqueous	***Anopheles, Culex, Aedes* spp.**	[105]
Feronia elephantum	Leaves	Aqueous		[106]
Chomelia asiatica	Leaves	Aqueous		[107]
Annona muricata	Leaves	Aqueous		[108]
Piper longum	Leaves	Aqueous		[110]
Anisomeles indica	Leaves	Aqueous		[111]
Clerodendrum chinense	Leaves	Aqueous		[112]
Holarrhena antidysenterica	Bark	Aqueous	***Culex, Aedes* spp.**	[109]
Ocimum canum	Leaves	Aqueous	***Hyalomma***	[115]

Table 3. List of parasites affected by AgNPs.

Parasite	Stage	Study	References
***T. gondii* (RH)**	Tachyzoites	*In vitro*	[40-42, 45-47]
	Tachyzoites	*In vivo* (Albino mice, BALB/c mice)	[39, 44]
***T. gondii* (ME49)**	Tachyzoites, bradyzoites	*In vitro*	[43]
P. falciparum	Trophozoites, rings	*In vitro*	[48, 50-55]
P. berghei	NI	*In vivo* (Albino mice)	[52]
P. chabaudi	NI	*In vivo* (BALB/c mice)	[56]
L. major	Amastigotes, promastigotes	*In vitro*	[57, 62, 63, 73]
	Amastigotes	*In vivo* (BALB/c mice)	[64, 65, 72]
	Amastigotes, promastigotes	*In vitro, in vivo* (BALB/c mice)	[74]
L. donovani	Amastigotes, promastigotes	*In vitro*	[58, 61, 67]
L. amazonensis	Amastigotes, promastigotes	*In vitro*	[59, 61, 66, 69-71]
	Amastigotes, promastigotes	*In vitro, in vivo* (BALB/c mice)	[60]
	Promastigotes	*In vitro*	[68]
Trypanosma* spp.	NI	*In vitro*	[41, 42]
T. cruzi	Epimastigotes	*In vitro*	[75]
G. lamblia	Trophozoites, cysts	*In vivo* (Rats)	[88]
A. castellanii	Trophozoites, cysts	*In vitro*	[76-79, 80, 82-84]
N. fowleri	Trophozoites	*In vitro*	[80, 85]
E. histolytica	Trophozoites, cysts	*In vitro*	[86, 87]
C. parvum	Oocysts	*In vitro, in vivo* (Mice)	[86, 89]
C. cayetanensis	Oocysts	*In vitro, in vivo* (Albino mice)	[90]
B. hominis	Non cyst forms	*In vitro*	[91]
E. granulosus	Protoscolices	*In vitro*	[92, 93]
F. hepatica	Eggs	*In vitro*	[94]
T. spiralis	Larvae	*In vitro*	[95]
B. malayi	Microfilaria	*In vitro*	[96]

NI: Not identified; ***:** *T. congolense, evansi, brucei*.

Table 4. List of arthropods affected by AgNPs.

Parasite	Stage	Study	References
An. stephensi	1st - 4th instar larvae, pupae, adults	*In vitro*	[51, 98-102, 103, 105-108, 110, 113, 114]
An. subpictus	3rd, 4th instar larvae	*In vitro*	[97, 111, 112]
C. quinquefasciatus	2nd, 3rd, 4th instar larvae, adults	*In vitro*	[97, 99, 100, 105-110]
C. tritaeniorhynchus	3rd instar larvae	*In vitro*	[111, 112]
Ae. aegypti	3rd, 4th instar larvae, adults	*In vitro*	[103, 110]
Ae. albopictus	1st, 3rd instar larvae, pupae, adults	*In vitro*	[111, 112, 114]
Hyalomma marginatum	Larvae	*In vitro*	[115]

2. Silver NPs provided highly sensitive and specific tools for diagnosis of infection with *P. falciparum*, *P. vivax* and intestinal parasitic infections.
3. Silver NPs possess anti-parasitic potentials against a wide range of medically important parasites *in vitro* especially, *T. gondii*, *Plasmodium* and *Leishmania* species with harmless employment *in vivo*. However, further studies *in vivo* and on clinical basis should be carried out.
4. Silver NPs offered an efficient approach for prevention of transmission of parasitic diseases through promising vector control, obviously, *Anopheles, Aedes* and *Culex* mosquitoes.

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