Chapter 13
Role of Nanoparticles in Treatment of Human Parasites

M. E. Della Pepa, F. Martora, E. Finamore, M. Vitiello, M. Galdiero and G. Franci

Abstract Nanobiotechnology is an important field with many new applications. This opportunity has been greatly embraced by the medical research community in the continuous search of novel opportunities for improving disease diagnosis, drug design and delivery. Understanding the mechanisms of disease for the design of new drugs is not enough, and unfortunately, infectious diseases continue to be a major health burden worldwide. Since ancient times, metals and especially silver were known for their antibacterial effects, but these days available methodologies allow the further exploitation of metal in the form of nanoscale materials. Metal nanoparticles are attracting much interest because of their potent antibacterial activity, but many studies have also shown a meaningful activity of metal nanoparticles against viruses, fungi and parasites. This chapter aims to summarize emerging efforts for the application of metallic nanoparticles in the never-ending battle against parasitic diseases. We have focused on four of the major parasitic diseases that afflict millions of people worldwide, specifically malaria, leishmaniasis, trypanosomiasis and schistosomiasis. The failure to respond to the increasing demand for effective antiparasitic drugs made imperative to explore new avenues; therefore, metal and metal oxide nanoparticles seem to represent an excellent therapeutic alternative.

Keywords Metal nanoparticles · Parasites · Malaria · Leishmaniasis Trypanosomiasis · Schistosomiasis

Nomenclature

Ag	Silver
Ag_2O	Silver oxide
AgNPs	Silver NPs

M. E. Della Pepa · F. Martora · E. Finamore · M. Galdiero · G. Franci (✉)
Experimental Medicine Department, University of Campania "Luigi Vanvitelli", Naples, Italy
e-mail: gianluigi.franci@unicampania.it

M. Vitiello
Department of Clinical Pathology, Virology Unit, "San Giovanni di Dio e Ruggi d'Aragona Hospital", Salerno, Italy

M. Rai and C. Alves dos Santos (eds.), *Nanotechnology Applied To Pharmaceutical Technology*, https://doi.org/10.1007/978-3-319-70299-5_13

AgNPs	Silver NPs
AK	Arginine kinase
AmB	Amphotericin B
Au	Gold
AuNPs	Gold NPs
CaO	Calcium oxide
CL	Cutaneous leishmaniasis
CNS	Central nervous system
CQ	Chloroquine
CQ-r	CQ-resistant
CQ-s	CQ-sensitive
CuO	Copper oxide
DA	Dopamine
FDA	Food and drugs administration
FRET	Fluorescence energy transfer
GSH	Glutathione S-transferase
HBV	Hepatitis B virus
HIV-1	Human immunodeficiency virus type-1
HPIV-3	Human parainfluenza virus type 3
HSV-1	Herpes simplex virus type 1
HSV-2	Herpes simplex virus type 2
MA	Meglumine antimoniate
MDA	Malondialdehyde
MgO	Magnesium oxide
MgONPs	Magnesium oxide NPs
ML	Muco-cutaneous
MPV	Monkeypox virus
MRSA	Methicillin–resistant *Staphylococcus aureus*
MRSE	Methicillin–resistant *Staphylococcus* epidermidis
MTT	Tetrazolium salt colorimetric assay
NE	Norepinephrine
NO	Nitric oxide
NPs	Nanoparticles
PdNPs	Palladium NPs
PfCRT	*P. falciparum* chloroquine resistance transport
PfThzK	5-(2-hydroxyethyl)-4-methylthiazolekinase
PZQ	Praziquantel
ROS	Reactive oxygen species
SeNPs	Selenium nanoparticles
SiO_2	Silicon dioxide
TbAK	*Trypanosoma brucei* AK
TCRV	Tacaribe virus
TiO_2	Titanium dioxide
TiO_2Ag-NPs	Silver-doped titanium dioxide nanoparticles

13.1 Introduction

Despite the extensive advancement in medical technology, parasitic diseases still represent a tremendous threat for human health and life. Parasites are among the earliest organisms existing in nature and are present throughout the world.

Although the most deleterious human diseases provoked by parasites manifest in developing countries, they represent a thoughtful health risk in many developed areas. These days increased vulnerability to infectious diseases is a mainstay of our modern society where several factors have a profound effect. These factors include a significant increase in international tourism, immigration policies, the international trade of food products, the lack of public awareness and knowledge of parasites, the increasing number of immunosuppressed and ageing people. In spite of the never-stopping development of scientific knowledge in medical practice, parasitic diseases are still to be considered a problem with its payload of disability, death and distress. Parasites belong to two major taxonomic groups: protozoa and helminths.

Protozoa are microscopic single-celled eukaryotes with size range from 2 to 100 μm. These are constituted by a membrane-bound nucleus and organelles. In contrast, helminths are multicellular macroscopic organisms, generally worm-like displaying rather complex tissues differentiation and organs with large diversity in dimensions, from less than 1 mm to more than 1 m. Pathogenesis of human disease due to parasites is highly variable and is affected by: (i) route of exposure; (ii) presence of anatomical barriers; (iii) replication strategies; (iv) host cellular damage; (v) life-stage of parasite; and (vi) interaction with host defences.

A single parasite has the capability to cause both acute and chronic infections with incubation time that goes from months to years, or even illness with no apparent signs or symptoms.

In this scenario, immunosuppressed individuals and those with acquired immunodeficiency syndrome have higher risks of parasitic disease.

Although human parasites are endemic in the tropical and subtropical areas of the planet, because of the new epidemiological scenarios due to migrations, climate change and international travels, these phenomena are increasing even in those countries considered non-endemic ones (Norman et al. 2010).

The size of global impact of parasitic infections is often underestimated and under-perceived; however, literature data on the worldwide number of parasite-associated disabilities and deaths are stunning. Each year there are hundreds of millions of people infected with diseases-causing parasites, particularly in tropical and subtropical regions of the world, resulting in an estimated one million deaths (WHO 2013).

Despite the recent technological developments that have taken the discovery of antiparasitic vaccines a step further towards their realization, pharmacological treatment is still the only effective and relatively cheap strategy for parasitic

diseases control. Due to limited research investments in these fields, few chemotherapeutic agents were discovered and characterized in the last 30 years.

Novel targets are continuously investigated as antiparasitic targets for drug discovery with the attempt of: (i) reducing toxicity; (ii) developing easier administration protocols; (iii) allowing long-term storage even in extreme weather conditions; (iv) decreasing resistance development; (v) achieving cheaper manufacturing procedures; (vi) obtaining broad spectra of action against the various stages of parasite development; and (vii) promoting a faster effects.

Due to the fact that parasites are eukaryotic organisms, similar to architecture of human cells, drug selectivity is often limited and thus host toxicity is of paramount importance when considering safety use of such drugs. In fact, many antiparasitic agents act on cellular pathways shared between the pathogen and the host. For instance, many antiprotozoan drugs interfere with nucleic acid synthesis or carbohydrate metabolism, and many antihelmintic drugs act on shared neuromuscular system targets. However, treatment strategy consistently differs in antiprotozoan and antihelmintic infections. Most antihelmintic drugs target adult organisms in a non-proliferating phase. On the other hands, many antiprotozoan drugs target an earlier developmental stage with a higher proliferation rate.

Moreover, parasite-related factors such as multiple parasite developmental stages and chronic infection, or drug-related factors, such as short half-life, limited distribution or rapid metabolism, make the chemotherapy treatment of these diseases particularly complex and insidious.

Nowadays, in the prokaryotic world, chemoresistance is widely spread causing an emerging health problem as reported by the WHO. In this scenario, we are observing the same events for the antiparasitic agents with the complication that the resistance mechanism is still unknown.

For some of them, genetic mutation and selective pressure mediated by drugs on different species of parasites results in drug uptake decrease. The tip of the iceberg in antiparasitic resistance is palpable in the case of *Plasmodium* species, particularly for *P. falciparum*. In sub-Saharan Africa, Asia and Latin America, for instance, resistance-related problems are widely common. Chloroquine resistance, that is probably the most common form of drug resistance, is associated with a mutation in a transport molecule of the parasite digestive membrane called *P. falciparum* chloroquine resistance transport (PfCRT) that produces a significant reduction of drug accumulation in *Plasmodium* digestive vacuoles. Other point mutation within the parasite genome can similarly result in resistance to sulphadoxine-pyrimethamine and atovaquone-proguanil (the latter due to mutations in the cytochrome B gene) and reduced susceptibility to mefloquine, quinine and quinidine. In this scenario, the applications of nanotechnology represent an interesting opportunity for novel antiparasitic approaches.

Our chapter aims to illustrate the reported antiparasitic activities of metal and metal oxide nanoparticles for each single parasite.

13.2 Metal Nanoparticles as Novel Antimicrobial Weapons

Nanotechnology is an emerging technological field focusing on the controlled manipulation of matter within a dimensional scale below the micrometre. Metal-based nanoparticles are considered the most interesting and promising as novel 'antimicrobial agents'.

From a structural point of view, metal nanoparticles have at least one dimension in the range from 1 to 100 nm, but more importantly, as particle size decreases, the surface area-to-volume ratio greatly increases; therefore, modalities and amount of the interactions with microbial surfaces are facilitated and resolve in an exponential increases of their chemical and biological reactivity compared to the bulk material of origin.

Several types of nanoparticles, including various metal and metal oxides, have been developed and evaluated for their antimicrobial activity by different research groups; examples include silver (Ag), gold (Au), Ag oxide (Ag_2O), zinc oxide (ZnO), titanium dioxide (TiO_2), calcium oxide (CaO), copper oxide (CuO), magnesium oxide (MgO) and silicon dioxide (SiO_2) (Dizaj et al. 2015).

In recent years, scientific literature has highlighted several metal nanoparticles exhibiting antimicrobial activity against a broad range of micro-organisms (Dizaj et al. 2014).

Antibacterial activity of nanoparticles against gram-negative and gram-positive bacteria has been widely documented; as an example, among metal and metal oxide nanoparticles, silver exhibits a clear antimicrobial activity, and several studies depict its antimicrobial activity against drug-resistant pathogens such as *Klebsiella pneumoniae*, erythromycin-resistant *Streptococcus pyogenes*, methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Staphylococcus epidermidis* (MRSE), *Proteus vulgaris* and *Salmonella typhimurium* (Rai et al. 2012; Franci et al. 2015). Silver nanoparticles (AgNPs) have been the principal platform for most of the studies reported so far; for example, ZnO nanoparticles were found to inhibit *S. aureus*, *Bacillus subtilis*, *S. epidermidis* and the gram-negative bacteria *Escherichia coli*, *Serratia marcescens* (Ghule et al. 2006; Nair et al. 2009; Moghaddam et al. 2017). CuO nanoparticles have been deeply investigated and shown to possess minor antibacterial activity against *S. aureus*, *Enterococcus faecalis*, *B. subtilis*, *Pseudomonas aeruginosa*, *Shigella sonnei* and *E. coli* (Khan et al. 2017). On the other hand, a recent study has shown a great potential of antibacterial efficiency against different non-pathogenic and pathogenic bacteria (*Bacillus subtilis*, *E. coli*, *S. aureus* and *P. aeruginosa*) of a porous ceramic device containing copper (Klein et al. 2013). Also Au nanoparticles have been describes as antibacterial, but conflicting results have often been reported. Zhang Y et al. 2015a and Zhang X et al. 2015b recently reviewed the literature and come to the conclusion that Au nanoparticles are generally not highly bactericidal. However, coexisting chemicals not completely removed from Au nanoparticles during

synthesis could be the reason AuNPs exerts the bactericidal activity. Au nanoparticles can be employed as carriers or delivery vectors for antibiotics or other drugs, therefore, enhancing the bactericidal effect of the antibiotics. In fact, a common strategy to improve the efficiencies and reduce the required dose of antibiotics has become the coating of nanoparticles with different antibiotic drugs on their stable surface.

Equally relevant is the activity found against bacterial biofilm formation and maintenance exhibited by some metal and metal oxide nanomaterials, such as Ag, Au, Bi, ZnO and TiO_2 nanoparticles (Kwak et al. 2001; Roe et al. 2008; Lellouche et al. 2009). Among nanoparticles, silver and titanium dioxide ones also exhibited significant antifungal activity against human pathogenic fungi, such as *Candida* species, *Aspergillus* spp., *Fusarium* spp and *Penicillium* spp, which represent aetiological agents of fungal infections specially in immunocompromised patients (Esteban-Tejeda et al. 2009; Jain et al. 2009; Krishnaraj et al. 2012; Gopinath and Velusamy 2013). Also viruses have been deeply investigated for their susceptibility to metal nanoparticles, mainly silver and gold nanoparticles (Galdiero et al. 2011; dos Santos et al. 2014; Rai et al. 2014, 2016).

Most detailed studies have been reported for the activity of metal nanoparticles against human immunodeficiency virus type-1 (HIV-1) where different resolutions have been adopted (Au, Ag, Cu, etc.) (Elechiguerra et al. 2005; Sun et al. 2005; Bowman et al. 2008; Lara et al. 2010a, b; Mastro et al. 2010; Kesarkar et al. 2012; Vijayakumar and Ganesan 2012).

Furthermore, several studies have shown that metal nanoparticles act as potential antiviral agents against various viruses including influenza virus (Mehrbod et al. 2009; Xiang et al. 2011). Herpes simplex virus type 1 and type 2 (HSV-1 and HSV-2) (Baram-Pinto et al. 2009, 2010; Gaikwad et al. 2013; Hu et al. 2014), cytomegalovirus (DeRussy et al. 2014), coxsackievirus B3 (Salem et al. 2012), Tacaribe virus (TCRV) (Speshock et al. 2010), vaccinia virus (VACV) (Trefry and Wooley 2013), human parainfluenza virus type 3 (HPIV-3) (Gaikwad et al. 2013), hepatitis B virus (HBV) (Lu et al. 2008), monkeypox virus (MPV) (Rogers et al. 2008), adenovirus (Chen et al. 2013) and respiratory syncytial virus (Sun et al. 2008). However, investigation of exact mechanism for the action of these nanoparticles is very difficult due to variations in their synthesis methods and sizes, but a consensus plan has become available in recent times pointing out to the importance of the capping of the nanoparticles for both enhancing the antiviral activity and reducing the toxicity towards host cells.

While the antibacterial, antifungal, antiviral activities of metal and metal oxide nanoparticles have been extensively studied, a little effort has been made to determine interactions of these nanoparticles with human parasites. The failure to respond to the increasing demand for effective antiparasitic drugs, mainly due to the resistant strains, has made indispensable to venture into novel research paths aimed at deepening the molecular biology and biochemistry of the parasites themselves. In this context, metal and metal oxide nanoparticles seem to represent an excellent therapeutic alternative, since they show a pronounced selectivity for specific parasites' biomolecules, not shared with human host.

13.3 Malaria

According to the report of the WHO, there were 212 million new cases of malaria and 429,000 deaths worldwide only in 2015; 90% of cases and 92% of malaria deaths are concentrated in sub-Saharan Africa. In this world region, children under five years of age are particularly vulnerable; indeed, they represent the 70% of all malaria deaths. Notwithstanding the huge efforts by the international community, malaria remains an acute public health problem (WHO 2015).

By physician point of view, malaria is an acute feverish disease, spread by a bite from a female mosquito of the genus Anopheles and caused by a protozoan of the genus *Plasmodium.* There are five species of plasmodia that infect humans viz. *Plasmodium falciparum*, *P. knowlesi*, *P. vivax*, *P. ovale* and *P. malariae*. Among them, *P. falciparum* is strongly correlated with the highly number of deaths (Daily 2017).

The sexual and asexual cycles of plasmodia reproduction are completed in different host species. The sexual phase occurs within the gut of mosquitoes. While feeding on a vertebrate host, mosquitoes transmit the parasite as sporozoites (the infective forms) into the blood circulation. Within the vertebrate, the plasmodia reproduce asexually, first in the liver and then in erythrocytes. Asexual replication progresses through a series of stages (ring, trophozoite, schizont) that culminates in the rupture of the erythrocyte, releasing merozoites, which initiate another cycle of replication infecting other erythrocytes. Some merozoites also develop within erythrocytes into male and female gametocytes. If a mosquito ingests mature male and female gametocytes during a blood meal, the sexual reproductive cycle of malaria can be initiated.

The main antimalarial drugs belong to the following classes: (i) 4-methanolquinoline (quinine, quinidine, mefloquine); (ii) 4-aminoquinoline (chloroquine); (iii) 8-aminoquinoline (primaquine); (iv) antifolate compounds (proguanil, pyrimethamine, sulphadoxine, dapsone); and (v) artemisin compounds (artemisinins, artemether, artesunate).

The complete treatment of malaria requires the destruction of erythrocytic schizonts, hepatic schizonts and erythrocytic gametocytes. The first terminates the clinical attack, the second prevents relapse, and the third renders the patient non-infectious to Anopheles and thus breaks the cycle of transmission. In order to achieve these results, physicians have to use a multi-drug approach because single drugs are not able to accomplish all the three goals set.

Chloroquine, a 4-aminoquinoline, has been the most commonly used; it acts by inhibiting haemoglobin degradation. Because chloroquine-resistant strains of *P. falciparum* are present in all endemicity areas, alternative schizonticidal agents should be used. These include quinine/quinidine, antifolate-sulphonamide combinations, mefloquine and the artemisinins.

Unfortunately, resistance to all of these agents is increasing, particularly in Southeast Asia. For this reason, the most effective way to slow down the further development of drug-resistant strains of *P. falciparum* is to use one of the

artemisinins, which prevent gametocyte development, in combination with quinine/quinidine, antifolate-sulphonamide combinations, mefloquine. Strains of *P. malariae*, *P. ovale*, *P. vivax* remain sensitive to chloroquine and may be treated with this agent, which destroys their gametocytes (Rathore et al. 2005; Gardella et al. 2008). A new era of antimalarial approach is emerging by the use of nanoparticles alone and/or in combination with commonly used drugs and may represent an innovative therapeutic effort for malaria treatment (Benelli 2016; Rai et al. 2017). Mishra et al. (2013) biologically synthesized AgNPs with the scope of minimizing cost of synthesis and toxicity of products, using pure enzyme α-amylase or by using soluble proteins of Neem or Ashoka leaf extracts. Different size and shape of AgNPs were produced, specifically, the amylase-mediated AgNPs had a triangular or an hexagonal shape and dimensions between 22 and 44 nm, and meanwhile Neem and Ashoka leaf extracts produced spherical nanoparticles with a range of 2–8 and 5–20 nm, respectively.

These nanoparticles revealed to be considerably stable in paragon to nanoparticles prepared through other methods. AgNPs ability to inhibit the growth of *P. falciparum* in ex vivo human red blood cell culture was performed to assess their antiplasmodial activity potential (Mishra et al. 2013). The results were highly encouraging since growth inhibition was obtained with IC_{50} values of 3.75 μg/ml (amylase-produced AgNPs), 8 μg/ml (Ashoka-produced AgNPs) and 30 μg/ml (Neem-produced AgNPs), whereas plant extracts or amylase alone did not show any activity up to 40 μg/ml (Mishra et al. 2013). The mechanism behind this inhibition is still unclear, and further investigations are mandatory. Moreover, the different characteristic of different AgNPs depends by the fact that plant extract and their related metabolites could provide a microenvironment that can influence the physicochemical and biological properties of the NPs formed.

Starting from this study, several plants and other reducing agents have been recently employed to prepare metal nanoparticles, and many of them showed a good potential as antiplasmodial effectors, sometimes with potency higher or comparable to that of chloroquine (CQ)-based drugs. Also Panneerselvam et al. (2016) have utilized AgNPs to study the in vitro inhibition of *P. falciparum* and their mosquitocidal properties against the malaria vector, *Anopheles stephensi.* In their study, the reducing and capping agent used for the AgNPs was *Pteridium aquilinum* leaf extract, and growth inhibition was measured by fluorescence, where a higher AgNPs inhibition activity against *P. falciparum* of the AgNPs when compared to chloroquine was registered. AgNPs using the *P. aquilinum* leaf extract were evaluated against CQ-resistant (CQ-r) and CQ-sensitive (CQ-s) strains of *P. falciparum*. IC_{50} of *P. aquilinum* extracts were 62.04 μg/ml (CQ-s) and 71.16 μg/ml (CQ-r); *P. aquilinum*-synthesized AgNP achieved IC_{50} of 78.12 μg/ml (CQ-s) and 88.34 μg/ml (CQ-r). Moreover, these AgNPs have shown toxicity against the young instars of the malaria vector and are able to reduce fecundity and longevity of mosquito adults.

Even earthworms have been used for providing the reducing enzymes for producing AgNPs from the bulk material. In fact, the coelomic fluid of the earthworms has several properties that have been known in East Asia for thousands of years. In

this study, AgNPs were synthesized using *Eudrilus eugeniae* earthworms aqueous extract as a reducing and stabilizing agent (Jaganathan et al. 2016).

AgNP showed uniformly distributed spherical particles with size ranging from 4 to 10 nm. In in vitro antiplasmodial assays, the nanoparticles gave higher inhibition rates against *P. falciparum* than chloroquine.

A further method employed is the exploitation of seaweed-mediated synthesis of antiplasmodial drugs. Murugan et al. (2015, 2016), in two different papers, described antiplasmodial activity of a plant-mediated synthesis of AgNPs using a seaweed extract of *Ulva lactula*, commonly known as sea lettuce (Murugan et al. 2015) and *Codium tomentosum* (Murugan et al. 2016). Both extracts (*U. Lactuca* and *C. tomentosum*) were able to elicit antiplasmodial activity comparable with the one obtained with AgNPs produced by using them as a reducing and capping agents, and both were effective against CQ-resistant plasmodium strains. Overall, *C. tomentosum* and *U. lactuca* metabolites and green-synthesized AgNP have the potential to be considered candidates novel and effective tools in the fight against *Plasmodium* parasites and their mosquito vectors.

The metabolites deriving from the natural extracts used for generating the nanoparticles often surround AgNPs with a thin layer of capping organic material, improving the AgNP stability in solution (Dinesh et al. 2015). The AgNPs produced in *aloe vera* extract were evaluated for antimalarial activity against CQ-r and CQ-s strain of *P. falciparum* demonstrating an increased ability to inhibit the growth of both CQ-r and CQ-s strains of *P. falciparum*.

Rajakumar et al. (2015) switched the focus on in vivo model for the antiplasmodial activity evaluation of synthesized palladium nanoparticles (PdNPs). PdNPs were obtained by using *Eclipta prostata* aqueous leaf extract. The PdNPs were orally administrated to infected Swiss albino mice of NK65 strain of *P. berghei*, at a different dose level from 0 to 4 days. After incubation for 24 h, Giemsa-stained thin blood films were evaluated, and the percentage of inhibition of parasite growth was determined under a microscope, highlighting that PdNPs were able to reduce parasitaemia by 78.13% with an inhibitory concentration IC50 value of 16.44 mg/kg/body weight (Rajakumar et al. 2015).

Finally, since green-synthesized gold NPs (AuNPs) have been recently employed as a new tool against mosquito vectors of medical application, Subramaniam et al. (2016) investigated the role of AuNPs biosynthesized using a flower extract of *Couroupita guianensis* as reducing and stabilizing agent for testing them as antimalarial drugs. AuNPs were crystalline with a cubic geometry, and the mean size was between 29.2 and 43.8 nm. They found a multiple effectiveness of the AuNPs both against CQ-r strains of *P. falciparum* and for the control of malaria vectors (Subramaniam et al. 2016). Previously, Karthik et al. (2013) explored the antimalarial activity of AuNPs synthesized using a marine actinobacteria (*Streptomyces* sp. LK-3). In detail, they evaluated the effects of AuNPs in mice infected with *P. berghei* by referring to the histomorphological changes in spleens and livers. They have shown an enhanced role of the liver in clearing parasites in asplenic mice. Further studies are mandatory in order to validate these data, especially to elucidate the underlying mechanism.

One of the principle drawbacks of antimalarial drugs is the toxicity. In this scenario, the antimalarial drugs/treatments should have few or absence of side effect on the human host. This goal could be achieved by the recognition of specific parasitic target that are not present in human cells. In this vision, Yao et al. (2015) have assumed that humans cannot synthesize B group vitamins while the malarial parasite possesses a biosynthetic pathway. For instance, the thiamine metabolizing enzymes, responsible for the synthesis of this group vitamins, are absent in humans (Wrenger et al. 2005, 2006; Müller et al. 2010). The interaction of AgNPs with a specific thiazolekinase, the 5-(2-hydroxyethyl)-4-methylthiazolekinase (PfThzK) has been investigated as a possible target. AgNPs were incubated with purified PfThzK at several concentrations and different time points. The resulting data indicated that PfThzK activity resulted 80% lower after only 10 min of incubation with 10 μM AgNPs, and a reduction of 90% was reached within 30 min of incubation. Moreover, it was proposed that the main interaction of AgNPs with PfThzK occurs through two surface sulphur-bearing amino acids (Met^{1} and Cys^{206}), which are in positions freely available for interaction with AgNPs and at a certain distance from the active site of the enzyme, supporting the non-competitive inhibition by the AgNPs.

Finally, very recently, magnetic nanoparticles (MNPs) produced by *Magnetospirillum gryphiswaldense*, a magnetotactic bacteria, have been tested on CQ-r and CQ-s *P. falciparum*, dengue virus (DEN-2) and two of their main vectors, *Anopheles stephensi* and *Aedes aegypti*, respectively (Suresh et al. 2015; Murugan et al. 2017).

13.4 Leishmaniasis

Leishmaniasis is a deadly vector-borne disease caused by the genus *Leishmania*, a protozoa which is transmitted by a phlebotomine sandfly. The disease occurs in three major forms: cutaneous (CL), muco-cutaneous (ML) and visceral leishmaniasis (VL), also called kala-azar. It is estimated that leishmaniasis threatens 350 million people all over the world (den Boer et al. 2011), and VL is strongly associated with HIV infection (Monge-Maillo et al. 2014). In the midgut of the vector, *Leishmania* parasite exists in the form of promastigotes. Subsequently, when the sandfly bites the mammalian host during blood meal, the parasite promastigote is internalized by macrophages and transforms into amastigotes, which survive and multiply within them, infecting several organs especially spleen, liver and bone marrow (Freitas-Junior et al. 2012). Therefore, amastigotes living inside the macrophages constitute one of the principle targets in antileishmania treatment, but also a difficult target to reach for the antileishmania drugs (Gutiérrez et al. 2016).

For several decades, the pentavalent antimonial compounds have been considered the first-line drug against leishmaniasis. These drugs showed several flaws including toxicity, the need of multiple parenteral administrations and the onset of

parasitic resistance. In a second time, amphotericin B (AmB), complexed with deoxycholate salt, has been prescribed as an alternative, unfortunately showing renal toxicity and requiring long courses of parenteral administration (Tiuman et al. 2011). Paromomycin presents poor oral absorption and so has been employed as parental or topical formulations for VL and CL; meanwhile, miltefosine is the only orally available drug for the treatment of VL. However, long treatment periods are required, and resistance and teratogen toxicity have been reported in many clinical studies (Sindermann and Engel 2006; Sundar and Olliaro 2007). Up to now, the liposomal formulation of AmB is the only drug delivery system approved by the Food and Drugs Administration (FDA) and commercially available as AmBisome for the treatment of VL, resulting more effective than AmB (WHO 2010; McGwire and Satoskar 2014; Sundar and Chakravarty 2015).

Treatment failure rates and limitations are high, and this treatment regimen must be improved by developing new more efficient drugs. Use of nanotechnology has shown promising results, thanks to the greater efficiency of particles to be active on different molecular targets. Among these targets, the trypanothione metabolism enzymes, primary involved in survival of the *Leishmania* parasite, have been studied as possible targets for metallic compounds and metal oxide nanoparticles (Navarro et al. 2010). Baiocco et al. (2010) demonstrated the potential application of AgNPs encapsulated by ferritin molecules in inducing an antiproliferative effect on *L. infantum* since ferritins are phagocytized by the infected macrophages (Uchida et al. 2008), and silver is an effective trypanothione inhibitor. Few years later, AgNPs have been thoroughly studied by Allahverdiyev et al. (2011). It was hypothesized that since AgNPs induce the release of reactive oxygen species (ROS), could be used as an alternative agents in the inhibition of *Leishmania* parasites, since they are sensitive to ROS. In fact, these parasites are able to inhibit the enzymatic mechanism of producing ROS mediated by macrophages, in which they can survive, while they may not be able to inhibit ROS produced by AgNPs. Moreover, since silver ions together with UV have shown to act in synergy against several micro-organisms (Butkus et al. 2004; Kim et al. 2008), different concentration of AgNPs have also been tested in in vitro assays to investigate their effects on growth, metabolic activity and infectivity on *Leishmania tropica* parasites, both in the presence and in absence of UV light (Allahverdiyev et al. 2011). The authors demonstrated that AgNPs show antileishmanial effects, which are increased under UV light. This increment may be derived from the fact that the released silver ions may complex with enzymes through cysteine groups and with parasitic proteins, which under the UV irradiation could generate monosulphur radicals causing further damage within parasites to be added to that produced by silver ions alone (Allahverdiyev et al. 2011). Furthermore, they highlighted a loss of infectivity of *L. tropica* promastigotes when exposed to both AgNPs and UV light. Following this line, the same group of researchers published a second study based on the use of silver-doped titanium dioxide nanoparticles (TiO_2Ag-NPs) on *L. tropica* and *L. infantum* in different conditions (dark and visible light) (Allahverdiyev et al. 2013). Results showed that TiO_2Ag-NPs are able to inhibit the biological properties of the parasites of the two species in a dose-dependent manner, resulting in a decrease of

viability of *L. infantum* and *L. tropica* promastigotes of about 3- and 10-folds, respectively, in the dark. The exposure under visible light increased this effect up to 20-fold for each species. However, the concentrations of TiO_2Ag-NPs resulting effective against *Leishmania* promastigotes were also toxic for host macrophagic cells and only partially active on *Leishmania* amastigotes. The authors reputed that the inhibitory effects of TiO_2Ag-NPs may be related to a greater production of ROS during light exposure. The fact that non-visible light-exposed TiO_2Ag-NPs also possess an antileishmanial activity and can be useful in the treatment of VL, while visible light-exposed TiO_2Ag-NPs can be more promising to treat CL. In order to decrease toxicity linked to the effective concentrations of TiO_2Ag-NPs on *Leishmania* parasites, in a successive paper Abamor and Allahverdiyev (2016) proposed the use of TiO_2Ag-NPs in combination with *Nigella sativa* essential oil. To date, essential oils of *N. sativa* have been showed to possess considerable antimicrobial properties (Mahmoud et al. 2002; Hannan et al. 2008) and also inhibitory activity against *L. tropica* and *L. infantum* parasites (Mahmoudvand et al. 2015). Non-toxic concentrations of both agents were determined by a tetrazolium salt colorimetric assay (MTT) and, afterwards, tested in combination on promastigote and amastigote–macrophage culture systems of *L. tropica*. The addition of *N. sativa* oil to the metal nanoparticle enhanced the drug efficiency leading to a complete inhibition of amastigote survival within macrophages and, moreover, reduced the overall toxicity of both compounds. The obtained results point out to a synergistic effects and increased efficacies of non-toxic concentrations of TiO_2Ag-NPs and *N. sativa* oil. One of the mechanisms of action that can be hypothesized is that *N. sativa* oil can induce the disruption of cell membranes of *Leishmania* parasites and consequently increase the amounts of TiO_2Ag-NPs uptake within the parasite, and so they may exert a combination antileishmanial action.

Zahir et al. (2015), in another study, investigated the antileishmanial properties of silver and titanium dioxide nanoparticles produced by green synthesis using an aqueous leaf extract of *Euphorbia prostrata*. Several experiments were performed against *L. donovani*: (1) Alamar Blue and propidium iodide uptake assays to analyse antileishmanial activity against promastigotes, (2) Giemsa staining was instead used against intracellular amastigotes, (3) DNA fragmentation and cell cycle progression assays were finally used to confirm the antileishmanial effects of both nanoparticles. The results showed that AgNPs were more active against *Leishmania* parasites in comparison with the controls and TiO_2NPs. The nanoparticles, both synthesized using extracts of *E. prostata*, were spherical with an average size of about 12.82 nm in the case of AgNPs, and circular and irregular in shape and mostly aggregated with a medium size of 83.22 nm in the case of TiO_2NPs. The fact that TiO_2NPs tend to form aggregates, probably bring forth nanoparticles unable to be transported through the cellular bilayer of the parasites, reducing their effectiveness. Overall, the proposed mechanism of action of these green-synthesized AgNPs is induction of cell death in *L. donovani* by promastigote proliferation inhibition and induction of caspase-independent cell death, which is largely due to necrosis.

In vivo topical effects of different concentrations of AgNPs in the treatment of lesions from leishmaniasis were tested in an animal model of Balb/c mice infected with viable stationary-phase *L. major* promastigotes in the base of tail. The study showed a significant decrease in splenic parasite load, but not in mean lesion diameter (Nilforoushzadeh et al. 2012)

Other metals and metalloids besides silver and titanium have been investigated as putative compounds against *Leishmania.* AuNPs of average size of 15.07 nm were synthesized through a one-pot method using polyphenolic quercetin (Das et al. 2013). The obtained quercetin conjugated AuNPs were evaluated against leishmanial macrophage infections and showed to be highly efficacious against wild-type *L. donovani* (IC_{50} 15 ± 3 μM), but still of interest against sodium antimony gluconate-resistant (IC_{50} 40 ± 8 μM) and paramomycin-resistant (IC50 30 ± 6 μM) strains.

Commercial nanoparticles including AgNPs, AuNPs, TiO_2NPs, ZnONPs and MgONPs were tested in a single laboratory at concentrations of 200, 20 and 2 μg/mL against *L. major* cultured promastigotes (Jebali and Kazemi 2013). The infectivity of *L. major* was used to determine the percentage of infected macrophage following the treatment with nanoparticles and irradiation. All nanoparticles decreased infectivity, and the combination of UV light together with nanoparticles led to a further lowering of infectivity than darkness. The highest antileishmanial activity was detected for AgNPs followed by AuNPs. In the case of metal oxide nanoparticles, TiO_2NPs represented the principal inhibitor while MgONPs had the lowest property. The fact that metal nanoparticles in comparison with metal oxide nanoparticles possess a stronger antileishmanial activity may be derived by the oxidation ability of the metal nanoparticles able to lead to increased damages to parasitic membranes, enzymes and DNA. MgONPs, especially when coated with glucose, have also been shown to silence the expression of important genes (Cpb and GP63, both surface molecules necessary for the parasite cell integrity) of *L. major* at sub-toxic concentrations (Bafghi et al. 2015). Different ZnO biocompatible nanoparticles doped with different concentrations of Cu have been tested for their in vitro efficacy against *Leishmania* parasite (Nadhman et al. 2015). A dose and time-dependent in vitro antileishmanial activity against promastigote cells and in different light conditions (cells were exposed with nanoparticles to direct sunlight, tungsten light and dark for 15 min) was observed with no considerable toxicity to normal cells. The parasite-killing ability was only in the presence of visible light by the production of more abundant ROS directly correlated with higher oxidative stress, membrane permeability and induced programmed death of the leishmania cells. Further, the nanoparticles were capped with PEG-400 in order to stabilize them and to limit the eventual interactions of the OH groups of ZnONPs with the surrounding environment and also to obtain a better penetration of nanoparticles into leishmania cells (Nadhman et al. 2016). Using nanoparticles at 10 μg/mL concentration and 15-min exposure to direct sunlight, leishmanicidal activity reached 100% after 3 h. Lipid peroxidation is probably the main reason of disturbance of the biophysical properties of membranes that modify its characteristics, mainly affecting

membrane fluidity with the result of upsetting the membrane ability to function as a barrier, therefore, leading to leishmania cell death.

The role of biogenic selenium nanoparticles (SeNPs) was investigated, by Beheshti et al. (2013) alone and in combination with meglumine antimoniate (Glucantime, MA) by Mahmoudvand et al. (2014). The authors first examined the effects of these nanoparticles primarily on the proliferation of promastigote and amastigote forms of *L. major* by an in vitro assay, and subsequently, they also determined the preventive and therapeutic effects in BALB/c mice presenting CL. It was noticed that promastigote proliferation decreased in a time-dependent manner, with high toxicity after 72 h of exposure, because of parasite's DNA fragmentation. The in vivo studies in mice showed a significant reduction in the development of lesions if preventive administration of sub-toxic doses of SeNPs was effectuated or had a better healing effect when administered after the development of CL. Subsequently, Mahmoudvand et al. (2014) corroborated the inhibition data obtained by Beheshti et al. (2013) on *L. tropica* and assessed that SeNPs in combination with MA significantly reduced the proliferation rate of amastigotes as compared with controls.

Therefore, combination of nanoparticles with existing drugs has proved to be effective in increasing the antileishmanial activity and reducing dosage and toxicity of drugs, and this strategy proved to be promising also with AgNPs synthesized by using *Anethum graveolens* L. (dill) leaf extract as reducing agent in combination with miltefosine (50 μM AgNPs plus 12.5 μM miltefosine) (Kalangi et al. 2016), and with amphotericin B adsorbed on the surface of AgNPs produced using aqueous extract of *Isatis tinctoria* as a reducing and capping agent (Ahmad et al. 2016).

Finally, since thermotherapy is one of the physical modalities proposed for the treatment of CL (Asilian and Davami 2006), the combination of metallic NPs with thermotherapy using microwave (MW) radiation was also proposed. In particular Sazgarnia et al. (2013) experienced the efficacy of treatment of CL in the presence of AuNPs and MW irradiation on promastigote and amastigote forms of *L. major*. The results confirmed the hypothesis that the antileishmanial effect of MW irradiation was intensified in the presence of AuNPs, increasing promastigotes death and reducing amastigotes proliferation, representing a new promising approach to treat leishmaniasis in future.

13.5 Trypanosomiasis

Trypanosomes are responsible of severe human diseases which can be distinguished in two major forms of trypanosomiasis: the African and the American ones. Human African trypanosomiasis or sleeping sickness occurs in sub-Saharan African countries where the tsetse fly vector is endemic and transmits the disease (WHO 2017). The aetiological agent is the haemoflagellate parasite of the species *Trypanosoma brucei*, and in particular, the single *Trypanosoma brucei gambiense*

accounts for more than 98% of reported cases. Sleeping sickness affects millions of people and is often fatal if untreated, being a serious public health problem. African forms of trypanosomiasis present a simple biological cycle with trypomastigote (the infective stage) that reproduce in the host blood, lymph and spinal fluid and the epimastigote form present in the salivary gland of the fly vector, where it continues reproduction to the infective forms. It is responsible for chronic disease by involvement of central nervous system (CNS) after a prolonged disease duration.

The American trypanosomiasis, commonly known as 'Chagas disease', is caused by *Trypanosoma cruzi* and represents the most important parasitic disease in the Americas. Human disease is found mostly among children in Central and South America, and about 40% of infected patients develop the chronic disease that involved several organs, especially heart, brain and liver. Here the peculiar intracellular amastigote forms multiply by binary fission and can destroy the tissue and cause irreversible damages, leading to death.

The actual treatment of African trypanosomiasis is based on several drugs including suramin, pentamidine, melarsoprol and a combination of eflornithine and nifurtimox (Murthy et al. 2013); on the other hand, the treatment of Chagas disease is limited to two agents, benznidazole, a nitromidazole derivate and nifurtimox, a nitroheterocyclic compound, which have proven activity against the acute phase of the disease, but appears to be ineffective in chronic Chagas disease. Both treatments suffer from several limitations: a significant adverse effects; an elevated toxicity due to the fact that high drug doses are required to work in different body organs; and the difficulties in reaching the disseminated intracellular parasites resulting often ineffective in chronic infections.

The limitations, related to ineffective antitrypanosomal therapies together with unsuccessful efforts in developing vaccine, encouraged researchers in identified new specific molecular targets. Adeyemi and Whiteley (2013) in their work focused, for the first time, on the study of the interaction of silver and gold nanoparticles with the enzyme arginine kinase (AK). AK is a critical enzyme to the survival of trypanosomes and is not present in humans (Miranda et al. 2006), representing an ideal putative drug target. The authors assessed the inhibitory potential of these metal nanoparticles on the activity of *Trypanosoma brucei* AK (TbAK). AgNPs and AuNPs led to a decrement in enzymatic activity of 75 and 62%, respectively, in a non-competitive manner. Fluorescence energy transfer (FRET) studies have been employed to investigate the putative mechanism of action. FRET analyses demonstrated that AgNPs are able to bind to AK with a greater affinity than AuNPs, interacting with tryptophan residues of surface molecules. The binding pocket is close to the arginine substrate; therefore, when NPs interact with the thiolate group of cysteine, a decreasing overall phosphoryl transfer between ADP and ATP was observed (Adeyemi and Whiteley 2014).

Silver and gold NPs have also been studied for in vitro growth inhibition of *T. brucei gabiense* by Rahul et al. (2015). In particular, phytosynthesized Ag and Au NPs have been used as nanocarriers for prodigiosin and violacein, two bacterial pigments with demonstrated antimicrobial and antiparasitic activity and produced by *Serratia* spp. and *Chromobacterium violaceum*, respectively (Lopes et al. 2009;

Durán et al. 2010; Genes et al. 2011). Microbial pigment prodigiosin in combination with metal NPs showed to be more effective than violacein: it has been recorded a significant decrease in the IC_{50} values of about 3.6-folds without increase of cytotoxicity towards mammalian cells. The authors supposed a synergistic effect probably due to a particular adherence of prodigiosin to the NPs with a better release of the microbial pigment to the bloodstream parasite forms.

13.6 Schistosomiasis

Schistosomiasis, also known as 'bilharziasis' or 'snail fever', infects 240 million people worldwide. It is endemic in many tropical and subtropical countries without or scarce sanitation (Steinmann et al. 2006). In terms of impact, this disease is second only to malaria (King et al. 2005). Schistosomiasis represents a helminthic infection caused by different species of blood fluke, principally *Schistosoma mansoni*, *S. haematobium* and *S. japonicum*, which are associated with two major human schistosomiasis, the intestinal and urogenital forms. The parasites live in freshwater snails and the cercariae, the infectious forms of the parasite, break out from the snail and contaminate water. Once in the host body, the larvae develop into adult schistosomes which live in the blood vessels, where the females release eggs. Some of the eggs are passed out of the body in the faeces or urine to continue the parasite's lifecycle. Others become trapped in body tissues, causing immune reactions and progressive damage to organs. Schistosomiasis can induce hepatosplenomegaly, liver fibrosis and cirrhosis and can involve also kidney and brain.

The standard treatment for schistosomiasis relies almost exclusively on praziquantel (PZQ), and the alternative is oxamniquine (Utzinger and Keiser 2004; Doenhoff et al. 2008). To date, PZQ has also been widely used for preventive mass drug administrations, increasing the concern on the enlarged possibilities of resistance development. Alternative new effective drugs are needed, in order to prevent and control the spread of the disease.

The current literature concerning the use of metallic nanoparticles in the treatment of schistosomiasis is primarily directed to the study of the healing effects of such nanoparticles on liver, brain and kidney. Dkhil et al. (2015a), in their studies, analysed the effect of three doses of AuNPs (0.25, 0.5 and 1.0 mg/kg body weight) on the neurotoxicity induced by *S. mansoni* in the brain of infected mice. AuNPs were prepared by a chemical reduction method using sodium citrate, and the obtained AuNps were spherical with an average diameter of 20 ± 5 nm. The neurological implications can occur during primary infections causing important alteration in the amount of brain neurotransmitters and with marked histological injuries, such as neuronal loss, vacuolated cytoplasm. From the comparison with control mice, emerged that the treatment of infected mice with AuNPs induced an increase of norepinephrine (NE), dopamine (DA), nitric oxide (NO) and malondialdehyde (MDA) accompanied by the reduction in brain glutathione (GSH) level and the alleviation of the brain histological impairments. Similarly, in a subsequent

study, they employed AuNPs in the treatment of the hepatic injury induced by schistosomiasis in experimentally infected mice (Dkhil et al. 2015b). AuNPs presented several beneficial effects, ranging from the hepatic worms count reduction by 32, 49 and 64%, respectively, according to the three different doses, to egg density reduction in liver tissue. Moreover, a decrease in inflammatory cellular infiltration and a reduction in the granuloma diameter were also recorded. Similar results have been obtained also by using SeNPs (Dkhil et al. 2016a). The healing effect of AuNPs has also been investigated on the kidney of schistosome-infected mice, resulting in a regeneration in the renal tissue morphology, in a reduction of nitrite/nitrate and MDA levels, with a better effectiveness than PZQ control (Dkhil et al. 2016b). In conclusion, all these studies collectively supported the hypothesis that the treatment with metal nanoparticles reduced the extent of the histological disturbance in brain, liver and kidney, with substantial ameliorative effects on schistosomiasis-promoted oxidative stress that may be due to AuNPs and SeNPs capability in scavenge free radicals.

The direct effects of AgNPs on *S. japonicum* cercariae have been investigated by Cheng et al. (2013). The results showed an immediate dose-dependent response inducing cercarial tail-shedding and a decrease in cercarial secretion, while longer treatments led to the death of cercariae. Surprisingly, cercariae treated for 30 min with AgNPs at low concentrations were still able to infect hosts regardless of the damage to the tails. High concentrations (above 125 μg/ml) were necessary to totally eliminate cercarial infectivity.

Surely further studies are still needed to assess the mechanism of modulatory effects of these metal nanoparticles and their potential therapeutic effects.

13.7 Mechanism of Action

The mechanisms of antiparasitic effect of metal nanoparticles are not well known, but catalytic oxidation, binding to parasite's protein and cellular constituents and ion release are the principal proposed modes of action. In particular, photocatalytic production of ROS damages parasitic components as well as disturbs energy transduction pathways.

When eukaryotic cells are infected by intracellular pathogens like viruses or fungi or parasites, a higher production of ROS levels is induced as a defence mechanism to kill the invading microbes (Lodge and Descoteaux 2006). For example, *Leishmania* parasites are able to survive into macrophages by inhibiting ROS production activating enzymatic pathways (Mehta and Shaha 2006). Since metal nanoparticles are themselves able to induce further ROS production, the inhibition of ROS by *Leishmania* parasites is overwhelmed and the antiparasitic action ensues. Many metal nanoparticles have been proved to produce ROS under UV light or generate heat under IR light, and both ROS and heat induce cellular damages that lead to cell death. In general, metal nanoparticles show a stronger antiparasitic activity compared with metal oxide nanoparticles, and this effect is

probably due to an extra damage to membranes and other cellular structures following oxidation. Proteins and DNA serve as targets for metal nanoparticles which bind to sulphur or phosphorus and lead to deterioration of cell membrane, enzymes and DNA. Additionally, antiparasitic metal nanoparticles can impair parasites surface molecules which are involved in the infectious mechanism of parasites (Arvizo et al. 2010). Finally, ions release from metal nanoparticles interact with cysteine-containing proteins, thereby inhibiting protein functions (Song et al. 2006). Mechanisms of action known are summarized in Table 13.1.

Table 13.1 Details of metal and metal oxide nanoparticles and their mechanisms of action on parasites

Parasite	Metal NP composition Size/shape	Synthesis	Mechanism of action	References
Plasmodium falciparum	AgNPs 22–44 nm; triangular/hexagonal	Amylase enzyme	N/A	Mishra et al. (2013)
	AgNPs 2–8 nm; spherical	*Neem* plant extract	N/A	Mishra et al. (2013)
	AgNPs 5–20 nm; spherical	*Ashoka* plant extract	N/A	Mishra et al. (2013)
	AgNPs 35–65 nm; spherical	Pteridium aquilinum leaf extract	N/A	Panneerselvam et al. (2016)
	AuNPs 29.2–43.8 nm; spherical and oval	*Couroupita guianensis* flower extract	N/A	Subramaniam et al. (2016)
	AgNPs 5 nm	Tannic acid	PfThzK inhibitor	Yao et al. (2015)
	AgNPs 20–35 nm; cubical	*Ulva lactula* seaweed extract	N/A	Murugan et al. (2015)
	AgNPs 20–40 nm	*Codium tomentosum* seaweed extract	N/A	Murugan et al. (2016)
Plasmodium berghei	PdNPs 30–110 nm; mostly spherical	*Eclipta prostrata* leaf extract	N/A	Rajakumar et al. (2015)
	AuNPs 5–50 nm; polygonal	Marine actinobacteria	N/A	Karthik et al. (2013)
	DQ-nanoparticles <400 nm	Chemical	Enhanced efficacy of DQ	Wang et al. (2014)
Leishmania tropica	AgNps 10–40 nm; round-shaped	Chemical	ROS production	Allahverdiyev et al. (2011)

(continued)

Table 13.1 (continued)

Parasite	Metal NP composition Size/shape	Synthesis	Mechanism of action	References
Leishmania tropica/infantum	TiO_2Ag NPs 90 nm; spherical (AgNPs)/rectangular (TiO_2)	Chemical	ROS production	Allahverdiyev et al. (2013)
Leishmania donovani	AgNPs 12.82 nm; spherical TiO_2 NPs 83.22 nm; circular and irregular shape	*Euphorbia prostrata*	TR inhibitor	Zahir et al. (2015)
Leishmania major	SeNPs 80–220 nm; spherical	*Bacillus sp. MSh-1*	DNA fragmentation	Beheshti et al. (2013)
	AuNPs 40 nm	Chemical	ROS production	Sazgarnia et al. (2013)
Trypanosoma brucei	Ag NPs 4–9 nm; spherical	Chemical	TbAK inhibitor	Adeyemi and Whiteley (2013)
	AuNPs 7–22 nm; spherical	Chemical	TbAK inhibitor	Adeyemi and Whiteley (2013)
Schistosoma mansoni	AuNPs 10–15 nm; spherical	Chemical	Scavenge free radical	Dkhil et al. (2015a, b/2016b)
	SeNPs	Chemical	Scavenge free radical	Dkhil et al. (2016a)

13.8 Conclusion and Future Perspectives

The coming of the nanotechnology era, that could be considered the most important discovery platform of our days, has completely revised the field of medical treatments with a constant interest for novel nanotechnology-derived products. Metal nanoparticles and their oxides are one proposed tool for creating a new class of broad-spectrum antimicrobial agents. Metals have been extensively used for many centuries as antimicrobial agents in agriculture, health care and industry in view of their ability to exert toxic activity against micro-organisms, especially bacteria. Nanotechnology applied to metals has opened the path for the development of new nanomaterials and the modification of the already present biocidal characteristics by modifying their properties through the fine tuning of particle size, shape and distribution. In the last years, novel impetus has been registered towards the use of metal nanoparticles as antimicrobials, and this has been followed by the analysis of their effect also against parasites.

Collectively, from the examples related to the parasites described in the present chapter, it can be concluded that AgNPs and AuNPs have been more frequently

analysed, and this is not surprising since the largest amount of data available in literature describes antimicrobial activity of AgNPs against several class of microbes, namely bacteria, viruses and fungi. The second aspect is that a capping agent is usually very effective in increasing the microbicidal effect of metal nanoparticles. A further point that needs to be considered is the fact that parasites are generally multicellular individuals, much more complex than, for example bacteria, therefore cultivation methods are less available for the systematic application of in vitro antiparasitic drug discovery technologies. As consequence, studies are often preliminary and do not provide sufficient insights on the mechanism of action. Furthermore, parasites can present in different forms during their developmental stage, which hamper the possibility of a compound to be efficient in each of these stages. Nevertheless, it is clear that metal nanoparticles exert a certain degree of toxicity on parasites.

Metal nanoparticles definitely alter many biological conditions, including parasitic cell viability, proliferation and infectivity, but their use in clinical settings for topical or systemic administration is still inadequate because of their potential toxic effect on normal host cells such as macrophages, skin cells, blood cells and other cell types. Therefore, metal nanoparticles need to be conjugated with biological compounds to improve their binding to parasites or need to be capped with agents that limit their toxicity. It is imperative to understand the impact that novel metal nanoparticles may have on biological organisms and the environment to minimize any harmful effect on humans. Nowadays, many research studies are also giving special attention towards the study of toxicological aspects of the nanomaterials prior to their application. Some parameters are of interest for the toxicological point of view, and the concentrations of nanoparticles needed to kill parasites should not exceed concentrations that would seriously endanger physiological functions of eukaryotic cells. Therefore, a better understanding of their biodistribution/accumulation in living systems is needed, but there are still very few studies on possible adverse effects and toxicity in vivo. To have a better description of the in vitro and in vivo toxicological aspects of metal nanoparticles, see references (Ge et al. 2014; Taylor et al. 2014; Golbamaki et al. 2015; Hadrup et al. 2015; Zhang Y et al. 2015a; Zhang X et al. 2015b; Bahadar et al. 2016). Further research is required to provide the necessary warranties to allow a safe exploitation of the interesting in vitro antiparasitic properties of metal nanoparticles and their transfer to the clinical setting. The biological activity of metal nanoparticles is strictly related to their surface chemistry, size, size distribution, shape, particle morphology, particle composition, coating/capping, agglomeration and dissolution rate, particle reactivity in solution, efficiency of ion release; therefore, the type of reducing agents adopted for driving the synthesis of nanoparticles is a crucial element for the overall cytotoxicity of each nanoparticle. Furthermore, the physicochemical properties of metal nanoparticles augment the bioavailability of the antiparasitic agents in both the systemic and local route controlling tissue distribution, cellular uptake and penetration through biological barriers (Jo et al. 2015).

References

Abamor ES, Allahverdiyev AM (2016) A nanotechnology based new approach for chemotherapy of Cutaneous Leishmaniasis: TIO2@AG nanoparticles—*Nigella sativa* oil combinations. Exp Parasitol 166:150–163

Adeyemi OS, Whiteley CG (2013) Interaction of nanoparticles with arginine kinase from *Trypanosoma brucei*: Kinetic and mechanistic evaluation. Int J Biol Macromol 62:450–456

Adeyemi OS, Whiteley CG (2014) Interaction of metal nanoparticles with recombinant arginine kinase from *Trypanosoma brucei:* Thermodynamic and spectrofluorimetric evaluation. Biochem Biophys Acta 1840:701–706

Ahmad A, Wei Y, Syed F, Khan S, Khan GM, Tahir K, Khan AU, Raza M, Khan FU, Yuan O (2016) *Isatis tinctoria* mediated synthesis of amphotericin B-bound silver nanoparticles with enhanced photoinduced antileishmanial activity: a novel green approach. J Photochem Photobiol B 161:17–24

Allahverdiyev AM, Abamor ES, Bagirova M, Ustundag CB, Kaya C, Kaya F, Rafailovich M (2011) Antileishmanial effect of silver nanoparticles and their enhanced antiparasitic activity under ultraviolet light. Int J Nanomed 6:2705–2714

Allahverdiyev AM, Abamor ES, Bagirova M, Baydar SY, Ates SC, Kaya F, Kaya C, Rafailovich M (2013) Investigation of antileishmanial activities of Tio2@Ag nanoparticles on biological properties of *L. tropica* and *L. infantum* parasites, in vitro. Exp Parasitol 135:55–63

Arvizo RR, Miranda OR, Thompson MA, Pabelick CM, Bhattacharya R, Robertson JD, Rotello VM, Prakash YS, Mukherjee P (2010) Effect of nanoparticle surface charge at the plasma membrane and beyond. Nano Lett 10:2543–2548

Asilian A, Davami M (2006) Comparison between the efficacy of photodynamic therapy and topical paromomycin in the treatment of Old World cutaneous leishmaniasis: a placebo-controlled, randomized clinical trial. Clin Exp Dermatol 31:634–637

Bafghi AF, Daghighi M, Daliri K, Jebali A (2015) Magnesium oxide nanoparticles coated with glucose can silence important genes of *Leishmania major* at sub-toxic concentrations. Colloids Surf B 136:300–304

Bahadar H, Maqbool F, Niaz K, Abdollahi M (2016) Toxicity of nanoparticles and an overview of current experimental models. Iran Biomed J 20:1–11

Baiocco P, Ilari A, Ceci P, Orsini S, Gramiccia M, Di Muccio T, Colotti G (2010) Inhibitory effect of silver nanoparticles on Trypanothione reductase activity and *Leishmania infantum* proliferation. ACS Med Chem Lett 2:230–233

Baram-Pinto D, Shukla S, Perkas N, Gedanken A, Sarid R (2009) Inhibition of herpes simplex virus type 1 infection by silver nanoparticles capped with mercaptoethane sulfonate. Bioconjug Chem 20:1497–1502

Baram-Pinto D, Shukla S, Gedanken A, Sarid R (2010) Inhibition of HSV-1 attachment, entry, and cell-to-cell spread by functionalized multivalent gold nanoparticles. Small 6:1044–1050

Beheshti N, Soflaei S, Shakibaie M, Yazdi MH, Ghaffarifar F, Dalimi A, Shahverdi AR (2013) Efficacy of biogenic selenium nanoparticles against *Leishmania major*: in vitro and in vivo studies. J Trace Elem Med Biol 27:203–207

Benelli G (2016) Plant-mediated biosynthesis of nanoparticles as an emerging tool against mosquitoes of medical and veterinary importance: a review. Parasitol Res 115:23–34

Bowman MC, Ballard TE, Ackerson CJ, Feldheim DL, Margolis DM, Melander C (2008) Inhibition of HIV fusion with multivalent gold nanoparticles. J Am Chem Soc 130:6896–6897

Butkus MA, Labare MP, Starke JA, Moon K, Talbot M (2004) Use of aqueous silver to enhance inactivation of coliphage MS-2 by UV disinfection. Appl Environ Microbiol 70:2848–2853

Chen N, Zheng Y, Yin J, Li X, Zheng C (2013) Inhibitory effects of silver nanoparticles against adenovirus type 3 in vitro. J Virol Methods 193:470–477

Cheng Y, Chen X, Song W, Kong Z, Li P, Liu Y (2013) Contribution of silver ions to the inhibition of infectivity of *Schistosoma japonicum* cercariae caused by silver nanoparticles. Parasitology 140:617–625

Daily JP (2017) Malaria 2017: update on the clinical literature and management. Current infectious disease reports

Das S, Roy P, Mondal S, Bera T, Mukherjee A (2013) One pot synthesis of gold nanoparticles and application in chemotherapy of wild and resistant type visceral leishmaniasis. Colloids Surf B 107:27–34

den Boer M, Argaw D, Jannin J, Alvar J (2011) Leishmaniasis impact and treatment access. Clin Microbiol Infect 17:1471–1477

DeRussy BM, Aylward MA, Fan Z, Ray PC, Tandon R (2014) Inhibition of cytomegalovirus infection and photothermolysis of infected cells using bioconjugated gold nanoparticles. Sci Rep 4:5550

Dinesh D, Murugan K, Madhiyazhagan P, Panneerselvam C, Kumar PM, Nicoletti M, Jiang W, Benelli G, Chandramohan B, Suresh U (2015) Mosquitocidal and antibacterial activity of green-synthesized silver nanoparticles from *Aloe vera* extracts: towards an effective tool against the malaria vector *Anopheles stephensi*? Parasitol Res 114:1519–1529

Dizaj SM, Lotfipour F, Barzegar-Jalali M, Zarrintan MH, Adibkia K (2014) Antimicrobial activity of the metals and metal oxide nanoparticles. Mater Sci Eng C Mater Biol Appl 44:278–284

Dizaj SM, Mennati A, Jafari S, Khezri K, Adibkia K (2015) Antimicrobial activity of carbon-based nanoparticles. Adv Pharm Bull 5:19–23

Dkhil MA, Bauomy AA, Diab MS, Wahab R, Delic D, Al-Quraishy S (2015a) Impact of gold nanoparticles on brain of mice infected with *Schistosoma mansoni*. Parasitol Res 114:3711–3719

Dkhil MA, Bauomy AA, Diab MS, Al-Quraishy S (2015b) Antioxidant and hepatoprotective role of gold nanoparticles against murine hepatic schistosomiasis. Int J Nanomed 10:7467–7475

Dkhil MA, Bauomy AA, Diab MS, Al-Quraishy S (2016a) Protective role of selenium nanoparticles against *Schistosoma mansoni* induced hepatic injury in mice. Biomed Res 27:214–219

Dkhil MA, Khalil MF, Bauomy AA, Diab MS, Al-Quraishy S (2016b) Efficacy of gold nanoparticles against nephrotoxicity Induced by *Schistosoma mansoni* infection in mice. Biomed Environ Sci 29:773–781

Doenhoff MJ, Cioli D, Utzinger J (2008) Praziquantel: mechanisms of action, resistance and new derivatives for schistosomiasis. Curr Opin Infect Dis 21:659–667

dos Santos CA, Seckler MM, Ingle AP, Gupta I, Galdiero S, Galdiero M, Gade A, Rai M (2014) Silver nanoparticles: therapeutical uses, toxicity, and safety issues. J Pharm Sci 103:1931–1944

Durán M, Faljoni-Alario A, Durán N (2010) *Chromobacterium violaceum* and its important metabolites–review. Folia Microbiol 55:535–547

Elechiguerra JL, Burt JL, Morones JR, Camacho-Bragado A, Gao X, Lara HH, Yacaman MJ (2005) Interaction of silver nanoparticles with HIV-1. J Nanobiotechnol 3:6

Esteban-Tejeda L, Malpartida F, Esteban-Cubillo A, Pecharromán C, Moya JS (2009) The antibacterial and antifungal activity of a soda-lime glass containing silver nanoparticles. Nanotechnology 20:085103

Franci G, Falanga A, Galdiero S, Palomba L, Rai M, Morelli G, Galdiero M (2015) Silver nanoparticles as potential antibacterial agents. Molecules 20:8856–8874

Freitas-Junior LH, Chatelain E, Kim HA, Siqueira-Neto JL (2012) Visceral leishmaniasis treatment: what do we have, what do we need and how to deliver it? Int J Parasitol Drugs Drug Resist 2:11–19

Gaikwad S, Ingle A, Gade A, Rai M, Falanga A, Incoronato N, Russo L, Galdiero S, Galdiero M (2013) Antiviral activity of mycosynthesized silver nanoparticles against herpes simplex virus and human parainfluenza virus type 3. Int J Nanomed 8:4303–4314

Galdiero S, Falanga A, Vitiello M, Cantisani M, Marra V, Galdiero M (2011) Silver nanoparticles as potential antiviral agents. Molecules 16:8894–8918

Gardella F, Assi S, Simon F, Bogreau H, Eggelte T, Ba F, Foumane V, Henry MC, Kientega PT, Basco L, Trape JF, Lalou R, Martelloni M, Desbordes M, Baragatti M, Briolant S, Almeras L, Pradines B, Fusai T, Rogier C (2008) Antimalarial drug use in general populations of tropical Africa. Malaria J 7:124

Ge L, Li Q, Wang M, Ouyang J, Li X, Xing MM (2014) Nanosilver particles in medical applications: synthesis, performance, and toxicity. Int J Nanomed 9:2399–2407

Genes C, Baquero E, Echeverri F, Maya JD, Triana O (2011) Mitochondrial dysfunction in *Trypanosoma cruzi*: the role of *Serratia marcescens prodigiosin* in the alternative treatment of Chagas disease. Parasites Vectors 4:66

Ghule K, Ghule AV, Chen BJ, Ling YC (2006) Preparation and characterization of ZnO nanoparticles coated paper and its antibacterial activity study. Green Chem 8:1034–1041

Golbamaki N, Rasulev B, Cassano A, Marchese Robinson RL, Benfenati E, Leszczynski J, Cronin MT (2015) Genotoxicity of metal oxide nanomaterials: review of recent data and discussion of possible mechanisms. Nanoscale 7:2154–2198

Gopinath V, Velusamy P (2013) Extracellular biosynthesis of silver nanoparticles using *Bacillus sp* GP-23 and evaluation of their antifungal activity towards *Fusarium oxysporum*. Spectrochim Acta Part A Mol Biomol Spectrosc 106:170–174

Gutiérrez V, Seabra AB, Reguera RM, Khandare J, Calderón M (2016) New approaches from nanomedicine for treating leishmaniasis. Chem Soc Rev 45:152–168

Hadrup N, Sharma AK, Poulsen M, Nielsen E (2015) Toxicological risk assessment of elemental gold following oral exposure to sheets and nanoparticles—a review. Regul Toxicol Pharmacol 72:216–221

Hannan A, Saleem S, Chaudhary S, Barkaat M, Arshad MU (2008) Antibacterial activity of *Nigella sativa* against clinical isolates of methicillin resistant *Staphylococcus aureus*. J Ayub Med Coll Abbottabad 20:72–74

Hu RL, Li SR, Kong FJ, Hou RJ, Guan XL, Guo F (2014) Inhibition effect of silver nanoparticles on herpes simplex virus 2. Genet Mol Res 13:7022–7028

Jaganathan A, Murugan K, Panneerselvam C, Madhiyazhagan P, Dinesh D, Vadivalagan C, Aziz AT, Chandramohan B, Suresh U, Rajaganesh R, Subramaniam J, Nicoletti M, Higuchi A, Alarfaj AA, Munusamy MA, Kumar S, Benelli G (2016) Earthworm-mediated synthesis of silver nanoparticles: a potent tool against hepatocellular carcinoma, *Plasmodium falciparum* parasites and malaria mosquitoes. Parasitol Int 65:276–284

Jain J, Arora S, Rajwade JM, Omray P, Khandelwal S, Paknikar KM (2009) Silver nanoparticles in therapeutics: development of an antimicrobial gel formulation for topical use. Mol Pharm 6:1388–1401

Jebali A, Kazemi B (2013) Nano-based antileishmanial agents: a toxicological study on nanoparticles for future treatment of cutaneous leishmaniasis. Toxicol In Vitro 27:1896–1904

Jo DH, Kim JH, Lee TG, Kim JH (2015) Size, surface charge, and shape determine therapeutic effects of nanoparticles on brain and retinal diseases. Nanomedicine 11:1603–1611

Kalangi SK, Dayakar A, Gangappa D, Sathyavathi R, Maurya RS, Narayana Rao D (2016) Biocompatible silver nanoparticles reduced from *Anethum graveolens* leaf extract augments the antileishmanial efficacy of miltefosine. Exp Parasitol 170:184–192

Karthik L, Kumar G, Keswani T, Bhattacharyya A, Reddy BP, Rao KV (2013) Marine actinobacterial mediated gold nanoparticles synthesis and their antimalarial activity. Nanomedicine 9:951–960

Kesarkar RO, Oza G, Pandey S, Dahake R, Mukherjee S, Chowdhary A, Sharon M (2012) Gold nanoparticles: effective as both entry inhibitors and virus neutralizing agents against HIV. J Microbiol Biotechnol Res 2:276

Khan S, Ansari AA, Khan AA, Abdulla M, Al-Obaid O, Ahmad R (2017) In vitro evaluation of cytotoxicity, possible alteration of apoptotic regulatory proteins, and antibacterial activity of synthesized copper oxide nanoparticles. Colloids Surf B 153:320–326

Kim JY, Lee C, Cho M, Yoon J (2008) Enhanced inactivation of *E. coli* and MS-2 phage by silver ions combined with UV-A and visible light irradiation. Water Res 42:356–362

King CH, Dickman K, Tisch DJ (2005) Reassessment of the cost of chronic helmintic infection: a meta-analysis of disability-related outcomes in endemic schistosomiasis. Lancet 365:1561–1569

Klein TY, Wehling J, Treccani L, Rezwan K (2013) Effective bacterial inactivation and removal of copper by porous ceramics with high surface area. Environ Sci Technol 47:1065–1072

Krishnaraj C, Ramachandran R, Mohan K, Kalaichelvan PT (2012) Optimization for rapid synthesis of silver nanoparticles and its effect on phytopathogenic fungi. Spectrochim Acta Part A Mol Biomol Spectrosc 93:95–99

Kwak SY, Kim SH, Kim SS (2001) Hybrid organic/inorganic reverse osmosis (RO) membrane for bactericidal anti-fouling. Preparation and characterization of TiO_2 nanoparticle self-assembled aromatic polyamide thin-film-composite (TFC) membrane. Environ Sci Technol 35:2388–2394

Lara HH, Ayala-Nuñez NV, Ixtepan-Turrent L, Rodriguez-Padilla C (2010a) Mode of antiviral action of silver nanoparticles against HIV-1. J Nanobiotechnol 8:1

Lara HH, Ixtepan-Turrent L, Garza-Treviño EN, Rodriguez-Padilla C (2010b) PVP-coated silver nanoparticles block the transmission of cell-free and cell-associated HIV-1 in human cervical culture. J Nanobiotechnol 8:15

Lellouche J, Kahana E, Elias S, Gedanken A, Banin E (2009) Antibiofilm activity of nanosized magnesium fluoride. Biomaterials 30:5969–5978

Lodge R, Descoteaux A (2006) Phagocytosis of *Leishmania donovani* amastigotes is Rac1 dependent and occurs in the absence of NADPH oxidase activation. Eur J Immunol 36:2735–2744

Lopes SC, Blanco YC, Justo GZ, Nogueira PA, Rodrigues FL, Goelnitz U, Wunderlich G, Facchini G, Brocchi M, Duran N, Costa FT (2009) Violacein extracted from *Chromobacterium violaceum* inhibits *Plasmodium* growth *in vitro* and *in vivo*. Antimicrob Agents Chemother 53:2149–2152

Lu L, Sun RW, Chen R, Hui CK, Ho CM, Luk JM, Lau GK, Che CM (2008) Silver nanoparticles inhibit hepatitis B virus replication. Antiviral Ther 13:253–262

Mahmoud MR, El-Abhar HS, Saleh S (2002) The effect of *Nigella sativa* oil against the liver damage induced by *Schistosoma mansoni* infection in mice. J Ethnopharmacol 79:1–11

Mahmoudvand H, Shakibaie M, Tavakoli R, Jahanbakhsh S, Sharifi I (2014) *In vitro* study of leishmanicidal activity of biogenic selenium nanoparticles against iranian isolate of sensitive and glucantime-resistant *Leishmania tropica*. Iran J Parasitol 9:452–460

Mahmoudvand H, Tavakoli R, Sharififar F, Minaie K, Ezatpour B, Jahanbakhsh S, Sharifi I (2015) Leishmanicidal and cytotoxic activities of *Nigella sativa* and its active principle, thymoquinone. Pharm Biol 53:1052–1057

Mastro AM, Hardy AW, Boasso A, Shearer GM, Eddy CR Jr, Kub FJ (2010) Non-toxic inhibition of HIV-1 replication with silver–copper nanoparticles. Med Chem Res 19:1074–1081

McGwire BS, Satoskar AR (2014) Leishmaniasis: clinical syndromes and treatment. QJM: Int J Med 107:7–14

Mehrbod P, Motamed N, Tabatabaian M, Estyar RS, Amini E, Shahidi M, Kheiri MT (2009) *In Vitro* antiviral effect of "Nanosilver" on influenza virus. DARU J Pharm Sci 17:88–93

Mehta A, Shaha C (2006) Mechanism of metalloid-induced death in *Leishmania spp*.: role of iron, reactive oxygen species, Ca2+, and glutathione. Free Radic Biol Med 40:1857–1868

Miranda MR, Canepa GE, Bouvier LA, Pereira CA (2006) *Trypanosoma cruzi*: oxidative stress induces arginine kinase expression. Exp Parasitol 114:341–344

Mishra A, Kaushik NK, Sardar M, Sahal D (2013) Evaluation of antiplasmodial activity of green synthesized silver nanoparticles. Colloids Surf B 111:713–718

Moghaddam AB, Moniri M, Azizi S, Rahim RA, Ariff AB, Saad WZ, Namvar F, Navaderi M (2017) Biosynthesis of ZnO nanoparticles by a new *Pichia kudriavzevii* yeast strain and evaluation of their antimicrobial and antioxidant activities. Molecules 22

Monge-Maillo B, Norman FF, Cruz I, Alvar J, López-Vélez R (2014) Visceral leishmaniasis and HIV coinfection in the mediterranean region. PLoS Neglected Trop Dis 8:3021

Müller IB, Hyde JE, Wrenger C (2010) Vitamin B metabolism in *Plasmodium falciparum* as a source of drug targets. Trends Parasitol 26:35–43

Murthy S, Keystone J, Kissoon N (2013) Infections of the developing world. Crit Care Clin 29:485–507

Murugan K, Samidoss CM, Panneerselvam C, Higuchi A, Roni M, Suresh U, Chandramohan B, Subramaniam J, Madhiyazhagan P, Dinesh D, Rajaganesh R, Alarfaj AA, Nicoletti M, Kumar S, Wei H, Canale A, Mehlhorn H, Benelli G (2015) Seaweed-synthesized silver

nanoparticles: an eco-friendly tool in the fight against *Plasmodium falciparum* and its vector *Anopheles stephensi*? Parasitol Res 114:4087–4097

Murugan K, Panneerselvam C, Subramaniam J, Madhiyazhagan P, Hwang JS, Wang L, Dinesh D, Suresh U, Roni M, Higuchi A, Nicoletti M, Benelli G (2016) Eco-friendly drugs from the marine environment: spongeweed-synthesized silver nanoparticles are highly effective on *Plasmodium falciparum* and its vector *Anopheles stephensi*, with little non-target effects on predatory copepods. Environ Sci Pollut Res Int 23:16671–16685

Murugan K, Wei J, Alsalhi MS, Nicoletti M, Paulpandi M, Samidoss CM, Dinesh D, Chandramohan B, Paneerselvam C, Subramaniam J, Vadivalagan C, Wei H, Amuthavalli P, Jaganathan A, Devanesan S, Higuchi A, Kumar S, Aziz AT, Nataraj D, Vaseeharan B, Canale A, Benelli G (2017) Magnetic nanoparticles are highly toxic to chloroquine-resistant *Plasmodium falciparum*, dengue virus (DEN-2), and their mosquito vectors. Parasitol Res 116:495–502

Nadhman A, Nazir S, Khan MI, Ayub A, Muhammad B, Khan M, Shams DF, Yasinzai M (2015) Visible-light-responsive ZnCuO nanoparticles: benign photodynamic killers of infectious protozoans. Int J Nanomed 10:6891–6903

Nadhman A, Khan MI, Nazir S, Khan M, Shahnaz G, Raza A, Shams DF, Yasinzai M (2016) Annihilation of *Leishmania* by daylight responsive ZnO nanoparticles: a temporal relationship of reactive oxygen species-induced lipid and protein oxidation. Int J Nanomed 11:2451–2461

Nair S, Sasidharan A, Divya Rani VV, Menon D, Nair S, Manzoor K, Raina S (2009) Role of size scale of ZnO nanoparticles and microparticles on toxicity toward bacteria and osteoblast cancer cells. J Mater Sci—Mater Med 20(Suppl 1):S235–S241

Navarro JA, Sánchez J, Peñafiel-Verdú C, Buendía AJ, Altimira J, Vilafranca M (2010) Histopathological lesions in 15 cats with leishmaniosis. J Comp Pathol 143(4):297–302

Nilforoushzadeh MA, Shirani-Bidabadi LA, Zolfaghari-Baghbaderani A, Jafari R, Heidari-Beni M, Siadat AH, Ghahraman-Tabrizi M (2012) Topical effectiveness of different concentrations of nanosilver solution on *Leishmania major* lesions in Balb/c mice. J Vector Borne Dis 49:249–253

Norman FF, Pérez de Ayala A, Pérez-Molina JA, Monge-Maillo B, Zamarrón P, López-Vélez R (2010) Neglected tropical diseases outside the tropics. PLOS Neglected Trop Dis 4:762

Panneerselvam C, Murugan K, Roni M, Aziz AT, Suresh U, Rajaganesh R, Madhiyazhagan P, Subramaniam J, Dinesh D, Nicoletti M, Higuchi A, Alarfaj AA, Munusamy MA, Kumar S, Desneux N, Benelli G (2016) Fern-synthesized nanoparticles in the fight against malaria: LC/MS analysis of *Pteridium aquilinum* leaf extract and biosynthesis of silver nanoparticles with high mosquitocidal and antiplasmodial activity. Parasitol Res 115:997–1013

Rahul S, Chandrashekhar P, Hemant B, Bipinchandra S, Mouray E, Grellier P, Satish P (2015) In vitro antiparasitic activity of microbial pigments and their combination with phytosynthesized metal nanoparticles. Parasitol Int 64:353–356

Rai MK, Deshmukh SD, Ingle AP, Gade AK (2012) Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria. J Appl Microbiol 112:841–852

Rai M, Kon K, Ingle A, Duran N, Galdiero S, Galdiero M (2014) Broad-spectrum bioactivities of silver nanoparticles: the emerging trends and future prospects. Appl Microbiol Biotechnol 98:1951–1961

Rai M, Deshmukh SD, Ingle AP, Gupta IR, Galdiero M, Galdiero S (2016) Metal nanoparticles: the protective nanoshield against virus infection. Crit Rev Microbiol 42:46–56

Rai M, Ingle AP, Paralikar P, Gupta I, Medici S, Santos CA (2017) Recent advances in use of silver nanoparticles as antimalarial agents. Int J Pharm 526:254–270

Rajakumar G, Rahuman AA, Chung IM, Kirthi AV, Marimuthu S, Anbarasan K (2015) Antiplasmodial activity of eco-friendly synthesized palladium nanoparticles using *Eclipta prostrata* extract against *Plasmodium berghei* in Swiss albino mice. Parasitol Res 114:1397–1406

Rathore D, McCutchan TF, Sullivan M, Kumar S (2005) Antimalarial drugs: current status and new developments. Expert Opin Investig Drugs 14:871–883

Roe D, Karandikar B, Bonn-Savage N, Gibbins B, Roullet JB (2008) Antimicrobial surface functionalization of plastic catheters by silver nanoparticles. J Antimicrob Chemother 61:869–876

Rogers JV, Parkinson CV, Choi YW, Speshock JL, Hussain SM (2008) A preliminary assessment of silver nanoparticles inhibition of Monkey poxvirus plaque formation. Nanoscale Res Lett 3:129–133

Salem ANB, Zyed R, Lassoued MA, Nidhal S, Sfar S, Mahjoub A (2012) Plant-derived nanoparticles enhance antiviral activity against Coxsakie virus B3 by acting on virus particles and vero cells. Dig J Nanomater Biostruct 7:737–744

Sazgarnia A, Taheri AR, Soudmand S, Parizi AJ, Rajabi O, Darbandi MS (2013) Antiparasitic effects of gold nanoparticles with microwave radiation on promastigots and amastigotes of *Leishmania major*. Int J Hyperth 29:79–86

Sindermann H, Engel J (2006) Development of miltefosine as an oral treatment for leishmaniasis. Trans R Soc Trop Med Hyg 1:17–20

Song X, Zhang J, Li E, Lu N, Yin F (2006) Preparation and characterization of rare-earth bulks with controllable nanostructures. Nanotechnology 17:5584–5589

Speshock JL, Murdock RC, Braydich-Stolle LK, Schrand AM, Hussain SM (2010) Interaction of silver nanoparticles with Tacaribe virus. J Nanobiotechnol 8:19

Steinmann P, Keiser J, Bos R, Tanner M, Utzinger J (2006) Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. Lancet Infect Dis 6:411–425

Subramaniam J, Murugan K, Panneerselvam C, Kovendan K, Madhiyazhagan P, Dinesh D, Kumar PM, Chandramohan B, Suresh U, Rajaganesh R, Alsalhi MS, Devanesan S, Nicoletti M, Canale A, Benelli G (2016) Multipurpose effectiveness of *Couroupita guianensis*-synthesized gold nanoparticles: high antiplasmodial potential, field efficacy against malaria vectors and synergy with *Aplocheilus lineatus* predators. Environ Sci Pollut Res Int 23:7543–7558

Sun L, Singh AK, Vig K, Pillai S, Shreekumar R, Singh SR (2008) Silver nanoparticles inhibit replication of respiratory Sincytial virus. J Biomed Nanotechnol 4:149–158

Sun RW, Chen R, Chung NP, Ho CM, Lin CL, Che CM (2005) Silver nanoparticles fabricated in Hepes buffer exhibit cytoprotective activities toward HIV-1 infected cells. Chem Commun 40:5059–5061

Sundar S, Olliaro PL (2007) Miltefosine in the treatment of leishmaniasis: clinical evidence for informed clinical risk management. Ther Clin Risk Manag 3:733–740

Sundar S, Chakravarty J (2015) Investigational drugs for visceral leishmaniasis. Expert Opin Investig Drugs 24:43–59

Suresh U, Murugan K, Benelli G, Nicoletti M, Barnard DR, Panneerselvam C, Kumar PM, Subramaniam J, Dinesh D, Chandramohan B (2015) Tackling the growing threat of dengue: *Phyllanthus niruri*-mediated synthesis of silver nanoparticles and their mosquitocidal properties against the dengue vector *Aedes aegypti* (Diptera Culicidae). Parasitol Res 114:1551–1562

Taylor U, Rehbock C, Streich C, Rath D, Barcikowski S (2014) Rational design of gold nanoparticle toxicology assays: a question of exposure scenario, dose and experimental setup. Nanomedicine 9:1971–1989

Tiuman TS, Santos AO, Ueda-Nakamura T, Filho BP, Nakamura CV (2011) Recent advances in leishmaniasis treatment. Int J Infect Dis 15:525–532

Trefry JC, Wooley DP (2013) Silver nanoparticles inhibit vaccinia virus infection by preventing viral entry through a macropinocytosis-dependent mechanism. J Biomed Nanotechnol 9:1624–1635

Uchida M, Terashima M, Cunningham CH, Suzuki Y, Willits DA, Willis AF, Yang PC, Tsao PS, McConnell MV, Young MJ, Douglas T (2008) A human ferritin iron oxide nano-composite magnetic resonance contrast agent. Magn Reson Med 60:1073–1081

Utzinger J, Keiser J (2004) Schistosomiasis and soil-transmitted helminthiasis: common drugs for treatment and control. Expert Opin Pharmacother 5:263–285

Vijayakumar S, Ganesan S (2012) Gold nanoparticles as an HIV entry inhibitor. Curr HIV Res 10:643–646

Wang H, Li Q, Reyes S, Zhang J, Zeng Q, Zhang P, Xie L, Lee PJ, Roncal N, Melendez V, Hickman M, Kozar MP (2014) Nanoparticle formulations of decoquinate increase antimalarial efficacy against liver stage *Plasmodium* infections in mice. Nanomedicine 10:57–65

World Health Organization, WHO (2010) Control of the leishmaniasis. World Health Organ Tech Rep 949:22–26

World Health Organization, WHO (2013) Sustaining the drive to overcome the global impact of neglected tropical diseases. Second WHO report on neglected tropical diseases

World Health Organization, WHO (2015) http://www.who.int/mediacentre/news/releases/2016/malaria-control-africa/en/

World Health Organization, WHO (2017) http://www.who.int/mediacentre/factsheets/fs259/en/

Wrenger C, Eschbach ML, Müller IB, Warnecke D, Walter RD (2005) Analysis of the vitamin B6 biosynthesis pathway in the human malaria parasite *Plasmodium falciparum*. J Biol Chem 280:5242–5248

Wrenger C, Eschbach ML, Müller IB, Laun NP, Begley TP, Walter RD (2006) Vitamin B1 de novo synthesis in the human malaria parasite *Plasmodium falciparum* depends on external provision of 4-amino-5-hydroxymethyl-2-methylpyrimidine. Biol Chem 387:41–51

Xiang DX, Chen Q, Pang L, Zheng CL (2011) Inhibitory effects of silver nanoparticles on H1N1 influenza A virus *in vitro*. J Virol Methods 178:137–142

Yao J, van Marwijk J, Wilhelmi B, Whiteley CG (2015) Isolation, characterization, interaction of a thiazolekinase *(Plasmodium falciparum*) with silvernanoparticles. Int J Biol Macromol 79:644–653

Zahir AA, Chauhan IS, Bagavan A, Kamaraj C, Elango G, Shankar J, Arjaria N, Roopan SM, Rahuman AA, Singh N (2015) Green synthesis of silver and titanium dioxide nanoparticles using *Euphorbia prostrata* extract shows shift from apoptosis to G0/G1 arrest followed by necrotic cell death in *Leishmania donovani*. Antimicrob Agents Chemother 59:4782–4799

Zhang X, Li W, Yang Z (2015a) Toxicology of nanosized titanium dioxide: an update. Arch Toxicol 89:2207–2217

Zhang Y, Shareena Dasari TP, Deng H, Yu H (2015b) Antimicrobial activity of gold nanoparticles and ionic gold. J Environ Sci Health Part C Environ Carcinog Ecotoxicol Rev 33:286–327