INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
23–0232/2023/30–3–189–199
DOI: 10.17957/IJAB/15.2075
http://www.fspublishers.org

Review Article

Greener Nanoparticles: Potential Alternative Approach to Fight Protozoal Diseases

Bushra Hussain Shnawa[1*], Parwin Jalal Jalil[1,2] and Sara Omar Swar[3]
[1]*Department of Biology, Faculty of Science, Soran University, Soran 30802, Iraq*
[2]*Scientific Research Centre, Soran University, Soran 30802, Iraq*
[3]*College of Agricultural Engineering Sciences, Salahaddin University, Kurdistan, Iraq*
[*]For correspondence: bushra.shnawa@soran.edu.iq
Received 23 June 2023; Accepted 15 July 2023; Published 28 August 2023

Abstract

Protozoa are unicellular eukaryotes parasitic organisms that are the primary causes of global morbidities and mortalities, in particular in developing nations. Several of them cause zoonotic protozoal diseases, such as toxoplasmosis, Chagas' disease, babesiosis, giardiasis and leishmaniasis, which can produce dangerous infections, with asymptomatic animals being able of spreading sickness. At present, protozoa are cured with chemotherapeutic antiparasitic medicines, although resistance to these treatments has developed over time due to misuse. Recently, Nanoparticles (NPs) are confirmed to be a significant innovation in the therapy and manipulation of parasitic infections in this scenario. In the last years, there has been tremendous progress in the discipline of parasite control using nanomedicine. Furthermore, NPs synthesized in green methods have a significant effect against most of the protozoan parasites. Recent research showed that green-based synthesized silver and gold NPs have demonstrated promising outcomes in the treatment and management of several parasitic illnesses. Additionally, other nanoparticles including zinc and copper and their oxides nanoparticles also exhibited anti-protozoan effects in recent studies. However, there are no sufficient studies explaining the mechanism of nanoparticles against protozoa. These nanoparticles function in a variety of methods, including organism plasma membrane damage, DNA interruption and protein production suppression, besides free radical fabrication. These substances are also efficient against intracellular parasites. The objective of this review is to summarize the technologies utilized to create nanoparticles as well as their potential modes of action against protozoal parasites. Additionally, it focuses on all the recent updates about biosynthesized nanoparticles against protozoan. This pilot review was designed to cover the updated nano medication against protozoal diseases which hopes to develop a modern effective drug and vaccine. © 2023 Friends Science Publishers

Keywords: Nanoparticles; Antiprotozoal; Green fabrication; Plant extract; Drug resistance

Introduction

The parasites are eukaryotic organisms that live inside or on their hosts and rely on them for resources like nutrition, accommodation and safety (Pritt 2020). Parasites are categorized into three divisions including protozoa, beside helminths as well as ectoparasites based on the parasites' morphology, genetic variation, evolution and adaptations (Rokkas *et al*. 2021). Parasitic infections are a major cause of death in these illnesses, and the majority of these illnesses are regarded as neglected tropical diseases. Chemotherapeutic drugs and ethnobotanicals remedies have been used traditionally by people to treat certain parasite illnesses, particularly malaria and gastrointestinal parasitic infections as well as echinococcosis (Shnawa *et al*. 2017; Ebiloma *et al*. 2019; Belete 2020; Imarhiagbe 2021; Aslam *et al*. 2022; Nawaz *et al*. 2022). Furthermore, the secondary metabolites that give these therapeutic plants their anthelmintic potential include tannins, flavonoids and essential oils (Degla *et al*. 2022).

Parasite transmission happens *via* vectors, faecal-oral contamination, or direct connection (Imarhiagbe 2021). Both humans and animals are susceptible to parasite attacks, which constitute a serious threat to both of their lives. Because parasitic illnesses may not present any observable symptoms, they are more difficult to diagnose and cure than bacterial diseases (Shnawa 1995; Zaheer *et al*. 2021).

Protozoa include several pathogenic parasites that impact human and animal health like *Entamoeba histolytica*, *Toxoplasma gondii, Sarcosystis* spp. and others (Shani *et al*. 2012; Shnawa 2017; Swar and Shnawa 2020). Among these protozoan parasites, the most harmful and fatal parasite infecting humans is *Plasmodium falciparum* (White *et al.* 2014; Tabassum *et al*. 2022). Some antiprotozoal medications that are available and used to treat various protozoal illnesses are including antimalarials, anti-

amoebic, anti-giardial, trypanocidal, anti-leishmanial and anti-toxoplasmic medicines (Shibeshi *et al.* 2020; Syed *et al.* 2020; Sobhy *et al.* 2021; Stevens *et al.* 2022). The parasite DNA is disrupted, protein synthesis is inhibited, the parasite membrane is damaged and the protozoa are killed by these chemicals (Bahuguna and Rawat 2020). However, several of the available treatments against these protozoans are no longer effective owing to the progress of resistance by these pathogenic protozoa (Raj *et al.* 2020). In this aspect, antimalarial drug resistance refers to a parasite strain's capacity to persist and/or proliferate despite the administration and absorption of medication at doses that are equal to or higher than those typically advised. The parasite mutation rate, total parasite load, chosen drug strength and other factors are among those that encourage the development of resistance to currently available antimalarial drugs (Shibeshi *et al.* 2020).

In recent times, nanotechnology is emerged as a promising arena of multidisciplinary research because of its extensive use in various fields of science. Metal NPs such as silver and gold nanoparticles (NPs) are recommended for various illnesses as drugs (Patra *et al.* 2015; Xu *et al.* 2020). The production of NPs through green biosynthesis, which relies on plant extracts, is advised today. Aside from being simple, clean, efficient, safe, and affordable, the green production of metal NPs has many other benefits as well. According to recent results, using plants as an immune stimulant in *Oreochromis niloticus* culture systems represents a more affordable option than fish meal (Kiran *et al.* 2022).

As a result of NPs' exceptional characteristics, such as their large surface-to-mass ratio, quantum structures, and capacities to adsorb and transport other compounds (drugs, probes and proteins), nanoparticles are crucial for medical applications. Due to their ease of use, environmental friendliness, accessibility, and nontoxicity, the greener fabrication of metal oxide nanoparticles has garnered a lot of attention over the past few years. Also, nanoparticles are tested as anti-microbial substances to enhance the shelf life of food products (Shnawa *et al.* 2021; Azam *et al.* 2022; Shnawa *et al.* 2022a). The green process of NPs fabrication is depicted in Fig. 1.

Numerous studies on the development of nanotechnologies using a variety of plant extracts from various plant parts have been published (Kumar and Rajeshkumar 2018; Hano and Abbasi 2021).

Exosomes, liposomes, and solid lipid NPs, besides nano-vaccines, are examples of nano-sized particles used in the formulation of novel treatments and drugs carriers that have the potential to overcome problems with little bioavailability, reduced toxicity, sub-therapeutic drug accumulation in microbial reservoirs, in addition to, low patient adherence because of drug-correlated side effects and lengthy therapeutic schedules. Therapeutic methods based on nanotechnology provide a crucial tool in the battle against contagious protozoan diseases (Raghav *et al.* 2023).

The use of nanoparticle technology in the diagnosis, prognosis and treatment of diseases in people is known as nanomedicine. This field of study has the potential to revolutionize medical research. Nanomedicine applications include chemotherapeutic aspects, insulin pumps, diagnostic tests and a variety of medical sensors. They also include drug delivery systems for use in body tissues (Shnawa *et al.* 2022b).

Different diseases are caused by parasitic protozoan organisms. They are extremely complicated to control. The utilization of AuNPs and AgNPs against these protozoal diseases has been successful (Fig. 2). Below is a description of a few of these protozoans (Bajwa *et al.* 2022).

Globally, parasitic protozoan diseases are a major cause of mortality and morbidity. Tropical or non-endemic diseases spread because of numerous factors like climate change, extreme poverty, migration, and a lack of opportunities in life. Although there are many medications available to treat parasitic diseases, it has been noted that some strains are resistant to common medications (Gaona-López *et al.* 2023). Additionally, a lot of first-line medications have negative side effects that can range from mild to severe, including possible carcinogenic effects. To combat these parasites, new lead compounds are therefore required. Several studies were achieved regarding the antiparasitic potency, it is believed that nanoparticles play an essential role in vital aspects against these organisms within *in vitro* and *in vivo* models Therefore, it is anticipated that there will be significant growth in the field of using nanotechnology to combat these parasites. This review provides a concise summary of the main fabricated nanostructures, specifically biosynthesized ones and their potential as treatments for a group of medically significant protozoal diseases.

Updated nanoparticles research for controlling protozoal diseases

***Plasmodium* (malarial disease):** Malaria is a member of the greatest frequent tropical infections, which is brought on by parasite protozoa of the species *Plasmodium* spp. It is a significant public health issue with a value of 228 million patients with a great number of morbidity and mortality on a global scale (Ezzi *et al.* 2017). The rise of parasites that are resistant to medication, insufficient vector control methods, and a lack of malaria vaccines offer severe obstacles to the eradication of malaria. The drugs are frequently used in the medication and limitation of malaria, resulting in a variety of tissue damages, such as harmful toxic effects and the establishment of medication resistance (Al-Salahy *et al.* 2016; Gujjari *et al.* 2022).

Due to the various antimicrobial capabilities that have been assessed, research on silver nanoparticles (AgNPs) has lately intensified (Galatage *et al.* 2021). In an investigation into the antimalarial potential of gold (AuNPs) and silver (AgNPs) nanoparticles generated by *Syzygium jambos* (L.), Alston (Myrtaceae) leaf and bark extract. Both preparations

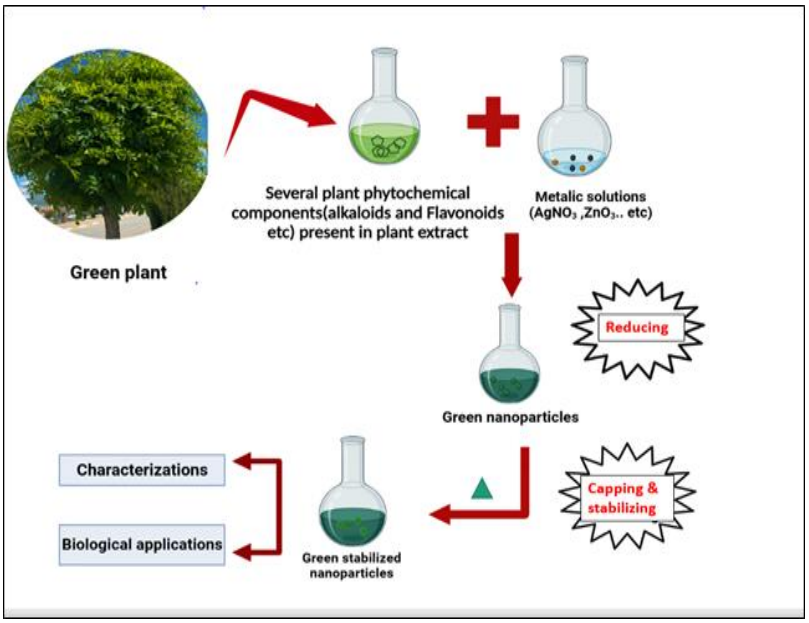

Fig. 1: Green fabrication of Nanoparticles

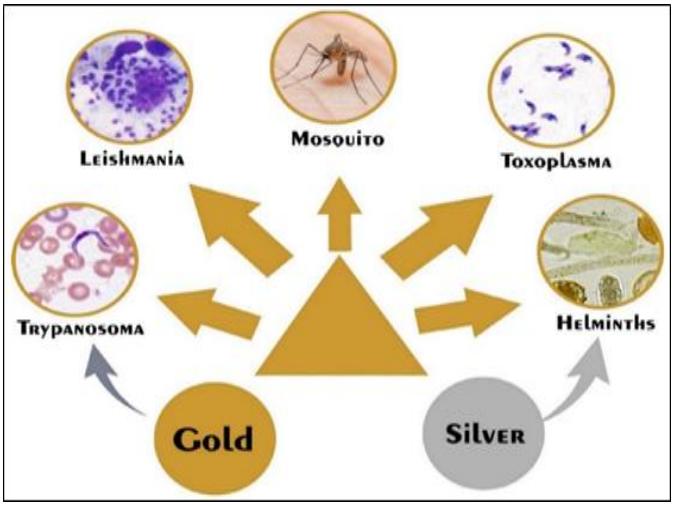

Fig. 2: Silver and gold nanoparticles' antiparasitic effects (Bajwa *et al.* 2022)

derived from AgNPs exhibited stronger antiplasmodial activity (Dutta *et al*. 2017).

Additionally, NPs created by *S. jambos* extracts showed minimal cytotoxicity towards rat skeletal muscle cell line (L6) and human cervical cancer cell line (HeLa), indicating their biocompatibility (Dutta *et al*. 2017). In a similar investigation, two *Artemisia* species, *A. abrotanum* and *A. arborescens*, were used to fabricate green-based silver nanoparticles (Ag NPs), which displayed hemocompatibility and antimalarial activity *in vitro* experiments on *Plasmodium falciparum* cells have been investigated. However, it was observed that *A. abrotanum*-AgNPs had stronger activity on pRBCs than *A. arborescens*-AgNPs. Additionally, the parasite growth was stopped in the ring stage after 24 and 48 h of *A. abrotanum*-AgNPs treatment, demonstrating the ability of these nanoparticles to prevent *P. falciparum* from maturing (Avitabile *et al*. 2020).

Also, AuNPs created by using the leaf extract from *Cymbopogon citrus* have demonstrated significant efficacy against a variety of mosquito species, including *Anopheles* (*A. stephensi*) (Mohammadi *et al.* 2021a). This evidenced that *plasmodim* spp. may be controlled by utilizing nanoparticles against mosquitos.

In another investigation, the effectiveness of neem-silver nitrate NPs utilizing watery extracts against two laboratory-adapted strains of *P. falciparum*— the 3D7 which is classified as (chloroquine-sensitive) and W2 known as

chloroquine-resistant was assessed. They verified that synthetic neem-AgNPs have a significant capability for usage in the medication of malaria. also, According to the hemolysis assay, both the aqueous extract and the synthesized neem-AgNPs do not have hemolysis action against healthy and parasitized erythrocytes (Ghazali *et al*. 2022).

Along the same line, adequate knowledge of the mechanisms of NPs that contribute to antiplasmodial activities is absent until now. Studies describing the biological mechanisms underlying Ag NPs' antiprotozoal activity are relatively rare (Sjöholm and Sandler 2019). Meanwhile, the study of AgNPs' antibacterial properties revealed important features of their action, which are mainly related to the production of reactive oxygen species, which promotes cell death mechanisms, particularly *via* mitochondrial apoptotic ways, along with significant cell membrane impairment and enzyme deactivation *via* silver binding (Huq *et al*. 2022). Moreover, Avitabile *et al*. (2020) explained that the main effects of nanoparticles include extensive cell membrane damage, enzyme deactivation *via* AgNPs binding, and the production of ROS (reactive oxygen species), which is a catalyst for cell death mechanisms, particularly *via* mitochondrial apoptotic pathways. All of these occurrences, singly or collectively, may be able to impair *plasmodium* cell functionality, producing effective results. According to their findings, the same authors concluded that the antiplasmodial activity of *Artemisia*-derived AgNPs *in vitro* tests against *P. falciparum* is promising (Avitabile *et al*. 2020).

In conclusion, malaria treatment and management depend mainly on chemical substances, which come with many drawbacks like serious toxic side effects, the emergence of drug resistance, and high medication costs. The development of new drugs is urgently required to overcome the clinical shortcomings of anti-malarial drugs. Nevertheless, the process of finding and developing new drugs is costly and time-consuming. Nanotechnological approaches could present promising alternatives, with increased efficacy and safety, for the treatment and control of malarial disease. Existing anti-malarial chemotherapeutic agents can be improved with nanotechnology-based preparations to achieve greater therapeutic benefits, safety, and cost-effectiveness, which improves patient agreement with treatment.

Leishmania (Leishmaniasis)

More than 20 different *Leishmania* species cause the zoonotic disease leishmaniasis, which is spread by more than 90 different phlebotomine sandflies, especially in low-income tropical nations. According to the World Health Organization, leishmaniasis causes 700,000 to 1 million new cases worldwide each year and up to 30,000 fatalities. The three main clinical manifestations of leishmania infection are cutaneous leishmaniasis, mucocutaneous leishmaniasis, and visceral leishmaniasis (WHO 2022a).

Nanotechnology has also been investigated as a potential innovation to treat these diseases because of the toxicity of the few therapeutic options available to treat neglected tropical diseases like leishmaniasis and Chagas disease. Green nanotechnology has recently enabled the development of various green nanoparticle treatment methods for leishmaniasis. In a study, the promastigote, as well as the amastigote form of *Leishmania major*, were treated in vitro with manufactured silver nanoparticles using ginger extract. Anti-amastigote assay results showed NPs' IC50 value was calculated to be 2.35mg.kg^{-1} after 72 h. Additionally, AgNPs significantly increased apoptosis and produced programmed cell death in promastigotes of *L. major* (Mohammadi *et al*. 2021b). They concluded that these nanoparticles may effectively reduce infected macrophages and that they caused the reduction of the proliferation rate of intramacrophage amastigotes. Based on these outcomes mentioned to treat *Leishmania* infections, these nanoparticles could be used as promising anti-leishmanial drugs. Similarly, another study utilizing a watery extract of *Eucalyptus camaldulensis* leaves was done, and the antileishmanial consequence of green synthesized AgNPs revealed a strong cytotoxic effect against *Leishmania tropica* at minimal doses (Zein *et al*. 2022).

The majority of reports suggest that the slow release of Ag ions, which damage the cell's surface while penetrating the cytoplasm and binding with the target locations, is what gives silver nanoparticles their antileishmanial capabilities. Additionally, reactive oxygen species can be created by silver nanoparticles. *Leishmania* is well recognized to be extremely sensitive to these oxygen species, making the medication, which may produce reactive oxygen species, an effective agent against *Leishmania* (Zein *et al*. 2022).

Awad *et al*. (2021) employed *Commiphora molmol* (myrrh) to synthesize Ag and Au NPs. Myrrh silver nanoparticles (MSNPs) were employed for subcutaneous lesions on mice infected by *L. major in vivo* and also in cultures (*in vitro*). MSNPs exhibited a concentration-dependent and when compared to chemical NPs and pentostam at the same doses, the MSNPs were considerably more efficient at the higher doses. Lesions healed completely *in vivo* after 21 days, but topical medications like pentostam and CNPs had little to no healing impact.

Due to NPs' spherical shape, they can enter cells (Kalangi *et al*. 2016) through phagocytosis, leading to the production of phagolysosomes in an acidified condition. AgNPs are subjected to oxidation, which releases free Ag^{+} ions that kill intracellular amastigotes. The oxidation of NPs is stimulated by the intracellular ROS that release by macrophages, which increases ROS production even more. AgNPs were able to activate macrophages to produce ROS, which significantly suppressed amastigote proliferation without killing the macrophages. In addition to the ROS,

phytochemicals (capping agents) generated from AgNPs may have favourable differential effects in infected and uninfected macrophages. These effects may improve the antileishmanial activity through their immunomodulatory effects by protecting the host cells (Lodge and Descoteaux 2006; Alti *et al*. 2020).

According to other recent research findings, silver nanoparticles with a curcumin coating may be a new, safe, and effective antileishmanial agent for the healing of cutaneous leishmaniasis (CL) infection. Increasing curcumin solubility and bioavailability is facilitated by the conjugate fabrication of curcumin and Ag NPs. The promastigotes and amastigotes of the protozoan parasite *L. major* were dramatically reduced by Curcumin AgNPs, confirming *in vitro* cell cytotoxicity and leishmanicidal action against parasites. During 48 h after infection, the NPs revealed strong antileishmanial action with IC50 values of 58.99 g/mL for promastigotes and EC50 values of 58.99 g/mL for amastigotes, without detrimental harmful toxicity on the murine macrophages. The CL lesion size was dramatically reduced in BALB/c mice that had been infected and were being treated with AgNPs (Badirzadeh *et al*. 2022).

Even though recently developed composite drugs based on the nanoparticles be successful, to combat the NPs' toxicity, bimetallic NPs made by reduction through greener plant extracts are suggested. Three different kinds of AU-Ag bimetallic nanoparticles were created in the study using fenugreek, coriander and soybean leaf extracts in a single reduction step. The antileishmanial effects of all three types of w bimetallic nanoparticles were very strong against promastigotes. The promastigotes experienced an apoptosis-like death brought on by the synthesized, bimetallic nanoparticles and the macrophages' antileishmanial activity was amplified. In macrophages, the number of intracellular amastigotes decreased by 31–46% (Alti *et al*. 2020).

It is crucial to look for new and effective medications that can treat this parasitic infection with few or no side effects given the rising number of leishmaniasis cases around the world, particularly cutaneous leishmaniasis. Recent research on green nanosynthesis has shown promise as a therapeutic strategy.

***Toxoplasma gondii* (Toxoplasmosis)**

Toxoplasma gondii is a worldwide distributed food-borne zoonotic protozoan parasite through frequent infection sources, that infects up to one-third of the population of humans (Shani *et al*. 2010). It causes noteworthy clinical signs, particularly in immunocompromised people, pregnant females, and cattle. Moreover, there is currently no reliable human vaccine available to combat this illness. Prophylaxis can therefore be recommended as the primary method of preventing toxoplasmosis (Chu and Quan 2021). Till now, chemotherapy using the drugs pyrimethamine and sulfadiazine is regarded as the "gold standard" therapy for toxoplasmosis owing to the absence of an efficient vaccination to avoid toxoplasmosis (Dunay *et al*. 2018). In recent years, different types of NPs have been used against *T. gondii* (Costa *et al*. 2020). More recently, nano vaccines have shown promise in preventing experimental toxoplasmosis in many experimental studies that have tested them (Brito *et al*. 2023).

In a study against chronic toxoplasmosis caused by *T. gondii* in mice, the anti-toxoplasmosis properties of green copper nanoparticles (CuNPs) alone and in combination with atovaquone were documented. The experimental groups had significantly fewer *T. gondii* tissue lesions than the control group, according to the results. CuNPs alone and in combination with atovaquone were found to be effective in preventing toxoplasmosis in mice (Albalawi *et al*. 2021). According to another study, AgNPs prevent bacterial growth and the development of biofilms while also reducing the viability of some parasitic species' cells (Vergara-Duque *et al*. 2020).

In addition, Mahmoodi *et al*. (2018) revealed that Cu NPs through the interaction with sulfhydryl groups(-SH) might lead to the denaturation of protein in bacteria. The findings of the other study by Chatterjee *et al*. (2014) showed that CuNPs may damage cell membranes as well as have numerous harmful consequences, including the oxidation of proteins, peroxidation of lipids, and the generation of ROS. To be linked in an *in vivo* research showed that cytokines enhance anti-*Toxoplasma* action in microglia *via* a NO-mediated mechanism (Halonen *et al*. 1998).

Furthermore, Albalawi *et al*. (2021) achieved quantitative real-time PCR to assess the mRNA levels of several cellular immunity mediators in mice infected with toxoplasmosis and treated with CuNPs, including IFN-γ, IL-12 and iNOs. In comparison to the control group, all mice in the experimental groups had higher levels of IFN-γ, IL-12, and iNO mRNA. Their outcomes showed that CuNPs alone and in combination with atovaquone had high efficacy in preventing mouse toxoplasmosis. CuNPs also have other benefits like enhanced cellular immunity and low toxicity, in addition to their preventative effects (Albalawi *et al*. 2021).

In addition, the Pretreatment of *Toxoplasma*-infected Balb/c mice with *Toxoplasma* with green fabricated AgNPs from *Phoenix dactylifera* and *Ziziphus spina-christi* through *in vivo* research led to reduced liver damage and improved histological characteristics. However, liver homogenate's antioxidant enzyme activity was markedly increased after nanoparticle treatment, which considerably reduced hepatic lipid peroxidation (LPO) and hepatic nitric oxide (NO) concentrations and proinflammatory cytokines. Additionally, the nanoparticle treatment decreased the immunoreactivity of hepatic tissues by regulating cytokines production like TGF-β and NF-B in the Balb/c mice (Alajmi *et al*. 2019).

According to previous research, these nanoparticles likely exert their antimicrobial properties *via* dual chief ways either through direct action *via* decreased cell permeability, inhibition of cell growth, and initiation of apoptosis and/or indirect impacts through the orientation of oxidative stress through the creation of H_2O_2 and the release of Zn ions, which allows them to penetrate cell walls and represent their toxic impacts (Khashan *et al*. 2020). Potential antiparasitic responses, particularly against toxoplasmosis, may result from increased synthesis of inflammatory cytokine in a variety of ways, containing higher expression levels of iNOs, ROS and NO synthesis and suppression of tryptophan within the cells, IFN-γ for instance, modifies cell metabolism, which can result in tryptophan deficiency in fibroblasts and iron starvation in enterocytes (Hunter and Sibley 2012; Chandrasekar *et al*. 2015).

The results of an experiment conducted recently by Saadatmand *et al*. (2021), showed that ZnNPs synthesized using *Lavandula angustifolia* extract have considerable therapeutic benefits against mice infected with chronic toxoplasmosis. Their conclusions showed that the number and the diameter of the tissue cysts in the brain of the infected mice significantly decreased after 14 days of oral treatment of Zn NPs. In the larger dose of Zn NPs taken orally, no *T. gondii* tissue cysts were seen. Compared to control groups, mice given 32.5, 75 and 150 mg/kg of ZnNPs for two weeks showed significantly higher expression levels of IFN-γ and iNOs. As it suggested that ZnONPs enhanced the innate immune system, which may account for its potent preventive benefits (Saadatmand *et al*. 2021).

As a result, the recent studies finding offer fresh insights into several natural plants that have historically been used to treat toxoplasmosis and other parasitic infections and may be helpful as a different mode of therapy for *T. gondii* infections as well as their greener-derived nanoparticles.

Trypanosoma (Trypanosomiasis)

Trypanosoma cruzi, the parasite that causes Chagas disease, is thought to infect 6 to 7 million people worldwide (WHO 2022b). Patients who are infected might show signs like hepatosplenomegaly, lymphadenopathy and facial edema. Nifurtimox and benznidazole which are nitro heterocyclic drugs with the mechanism of action of causing the formation of reactive oxygen species like superoxide radicals and hydrogen peroxide are used to treat Chagas disease. These reactive oxygen species subsequently result in oxidative damage to the parasite, which ultimately leads to its death. However, patients taking these medications run the risk of suffering from many serious side effects, including skin irritation, liver and pancreatic damage (Thakare *et al*. 2021; Fernandes *et al*. 2022).

In the aspect of targeting *T, cruzi*, Souza *et al*. (2022) extracted bioactive fucoidan from *Spatoglossum schröederi*, a brown seaweed. Then an environmentally friendly synthesis technique was used to create AgNPs that included fucan A (AgFuc). Regardless of the length of the treatment, AgFuc was found to be more effective than fucan A or silver at preventing parasites. AgFuc also caused 17% of parasites to die *via* apoptosis and 60% of parasites to die by necrosis. AgFuc, therefore, causes mitochondrial damage in the parasites, indicating that it has anti-*Trypanosoma cruzi* properties.

Moreover, by reducing the particle size, antibacterial AgNPs activity can be more effective. The particles are quite polydisperse, as can be seen from the AgFuc characterization, notably the UV-vis analysis and the size-dispersion histogram. As a result, the larger Ag fucan nanoparticles might not reach the parasites' cytoplasm, which could compromise AgFuc's ability to kill *Trypanosomes*. They recommended future research to focus on the perfect way for producing AgFuc NPs of a smaller size and assessing the impact of this on *T. cruzi* survival (Souza *et al*. 2022).

It is claimed that silver nanoparticles, which are produced from the bioactive polysaccharide xylan taken from corn cobs, have anti-*Trypanosoma* properties. Regardless of the length of the trial, the silver-xylan nanoparticles NX were more successful than benznidazole in reducing the ability of parasites (Brito *et al*. 2020).

Based on the IC50 value against *Trypanosoma evansi* in a study conducted by Rani *et al*. (2022), three naphthoquinone (NTQ) derivatives were chosen and gum damar was used to encapsulate them. All three NNTQs had a strong antitrypanosomal action and morphological alterations at 2–3 times lower drug concentrations, these nanopreparations produced more reactive oxygen species in the axenic culture of *T. evansi*. According to the findings, NNTQs increased ROS, apoptosis, and necrosis, which had a greater negative effect on *T. evansi*'s growth.

Furthermore, researchers integrated dual ideas: green chemistry and agro-waste valorization in a whole zero-waste procedure to solve pollution and neglected tropical disease topics. FM2-free Ag/Cu NPs were investigated for anti-kinetoplastid activity against two flagellates, *Leishmania* spp. and *T. cruzi*. The strongest leishmanicidal and trypanocidal effects were demonstrated by free Ag/Cu nanoparticles (Snoussi *et al*. 2022).

The gap in the mechanism of nanoparticles against protozoan has been investigated by Wang *et al*. (2021). African trypanosomiasis is caused by *Trypanosoma brucei*. Endocytosis of dual noble metal nanoclusters (NM-NCs), Ag2S-NC@MPA and AuNC@GSH, was studied in *T. brucei*. Both forms of NC can be efficiently taken up by *T. brucei* through a clathrin-dependent endocytosis route and demonstrated anti-parasitic activity by inducing pathological changes in apoptosis-related organelles. The Ag2S-NC@MPA primarily related to the proteins of the mitochondrion as well as the endoplasmic reticulum, whereas the AuNC@GSH primarily disrupted the biological action of cytoplasmic enzymes shared in mRNA maturation and signal transmission.

Table 1: The efficiency of different nanoparticles against the protozoal parasites

Nanoparticle	Source	Model	Parasite	Effect	Reference
AgFuc nanoparticles	Bioactive fucoidan from *Spatoglossum schröederi*	*in vitro*	*Trypanosoma cruzi*	-Regardless of the length of the treatment, AgFuc was more effective than fucan A or silver at preventing parasites from reducing MTT. -AgFuc also caused 17% of parasites to die *via* apoptosis and 60% of parasites to die by necrosis. -AgFuc causes mitochondrial damage in the parasites.	Souza *et al*. (2022)
AgNPs	Neem leaves *Azadirachta indica*	*in vitro*	*P. falciparum*	-Neem-AgNPs prevent P. falciparum from growing in infected human red blood cells. T -The hemolysis outcome demonstrates that AgNPs are non-toxic because their mean blood hemolysis rate (%) was 13%.	(Ghazali *et al*. 2022)
Gum damar-loaded naphthoquinone nanocapsules (NNTQ)	Gum damar	*in vitro*	*Trypanosoma evansi*	-Each of the three NNTQs shows a sizable antitrypanosomal impact as well as morphological alterations. -Compared to pure NTQs, the nanoformulations showed increased (ROS) generation in the axenic culture of *T. evansi* - Low cytotoxicity on horse peripheral blood mononuclear cells.	(Rani *et al*. 2022)
Ag/Cu NPs	sugarcane bagasse	*in vitro*	*L. donovani* & *L. amazonensis* and *T. cruzi*	-The Ag/Cu nanoparticles had strong leishmanicidal and trypanocidal effects, with IC50 values of 2.909 ± 0.051 for *L. donovani*, 3.580 ± 0.016 for *L. amazonensis*, and 3.020 ± 0.372 $mg.kg^{-1}$ for *T. cruzi*.	(Snoussi *et al*. 2022)
AgNPs	*Eucalyptus camaldulensis*	*in vitro*	*L. tropica*	-The highest inhibitory effect on parasites was at the highest concentration of AgNPs (3.75 g/mL), which resulted in a 90% reduction in parasite growth.	(Zein *et al*. 2022).
Curcumin-coated silver nanoparticle (Cur@AgNPs)	curcumin derived from turmeric	*in vitro* & *in vivo*	*L. major*	-An IC50 of 58.99 g/ml for promastigotes and an EC50 of 58.99 g/ml for amastigotes, the nanoparticle demonstrated strong antileishmanial activity. -The size of the (CL) lesion was dramatically reduced in the BALB/c mice that were being treated for infection with Cur@AgNPs.	(Badirzadeh *et al*. 2022)
CuNPs	*Capparis spinosa* fruit alone & combined with atovaquone	*in vivo*	Tehran strain *T. gondii*	- All experimental group mice showed higher mRNA for IFN-γ, IL-12, and iNO compared to the control group.	(Albalawi *et al*. 2021)
AgNPs	*Commiphora myrrha* (oelo-gum resins)	*in vitro* & *in vivo*	*L. major*	- At the higher concentrations 100, 150 l/100 l/100 ul showed a significant inhibitory effect for the MSNPS. - After receiving MSNP treatment in vivo, lesions were fully cured in 21 days.	Awad *et al*. (2021)
ZnNPs	*Lavandula angustifolia Vera*	*in vivo*	*T. gondii*	-Mice who had 32.5, 75, and 150 mg/kg of ZnNPs for two weeks showed significantly higher expression levels of IFN-γ and iNOs. -The average count of brain tissue cysts in the studied animals was significantly decreased using Zn NPs at 32.5 and 75 mg/kg for three weeks. -At 150 mg/kg for 14 days, no tissue cysts of *T. gondii* were seen in mice.	(Saadatmand *et al*. 2021)
AgNPs	Ginger rhizome extract	*in vitro*	*L. major*	-After one, two, and three days, the proliferation of *L. major* promastigotes was suppressed by Ag NPs at (40, 20, 10 and 5 $mg.kg^{-1}$). -There were reports of 7.3% and 32.2% viability % for macrophages and *L. major* promastigotes treated with the highest NPs amount (40 $mg.kg^{-1}$). -After 72 hours of incubation, the mean number of amastigotes in each macrophage was reduced by 1.25 and 2.5 $mg.kg^{-1}$ of Ag-NPs in comparison to control groups. -Additionally, after 72 hours of exposure, the IC50 value for this parasite strain of *L. major* was 2.35 $mg.kg^{-1}$.	(Mohammadi *et al*. 2021b)
silver-xylan nanoparticles (NX)	xylan, extracted from corn cob	*in vitro*	*Trypanosoma cruzi*	-The NX at 100 μg/mL cause 95% mortality of parasites through necrosis. -Although it displayed negligible cytotoxicity at 2000 μg/mL. -In 100 μg/mL the NX exhibit more effectiveness in affecting the parasites.	(Brito *et al*. 2020)
AgNPs	Two *Artemisia* species, *A. abrotanum* and *A. arborescens*	*in vitro*	*P. falciparum*	-Compared to *A. arborescens*- AgNPs, *A. abrotanum*-AgNPs exhibit higher antimalarial activity. -The smaller AgNPs have a more strong dose-dependent hemolytic impact. The small size of the NPs showed a distinctive impact on the parasite of *A. abrotanum*-AgNPs. -*A. abrotanum*-AgNPs were able to prevent the parasite from reaching its mature stage, keeping it in the ring stage.	(Avitabile *et al*. 2020)

Table 1: Continued

Table 1: Continued

Ag–Au BNPs	fenugreek, coriander, and soybean leaf extracts	*in vitro*	*Leishmania*	-Three different Au-Ag BNP types had IC50 values that ranged from 0.03 to 0.035 g/mL and demonstrated strong antileishmanial effects against promastigotes. -IC50 values for BNPs are significantly lower than those for miltefosine (IC50 = 10 g/mL). -The promastigotes experienced an apoptosis-like death brought on by the synthesized BNPs, and macrophages' antileishmanial activity was amplified. -In macrophages, the number of intracellular amastigotes was decreased by 31-46%.	(Alti *et al*. 2020)
AuNPs and AgNPs	Bark and leaf extract of *Syzygium jambos* (L.) Alston (Myrtaceae)	in vitro	*P. falciparum*	-AgNPs and AuNPs produced by both extracts exhibit great antiplasmodial activity against both chloroquine-sensitive (3D7) and resistant (Dd2) strains of *P. falciparum*. -NPs produced by *S. jambos* extracts were determined to be harmless to HeLa and L6 cell lines.	(Dutta *et al*. 2017)

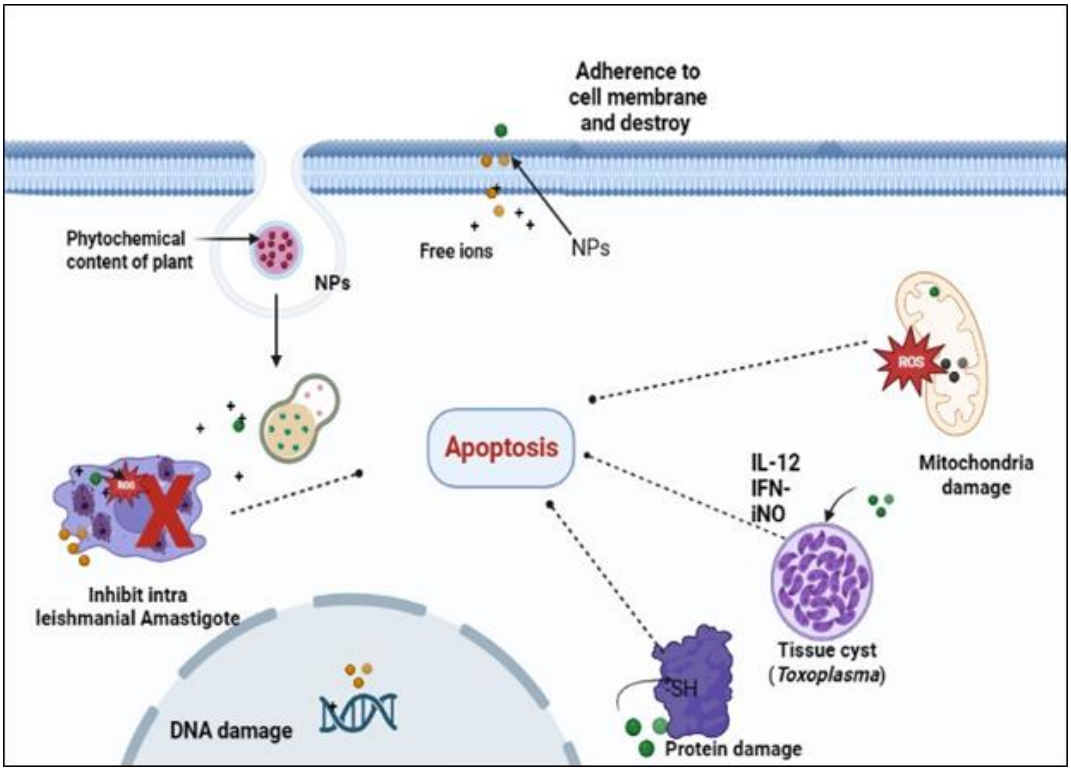

Fig. 3: Schematic illustration of the possible mechanisms of antiprotozoal activity of green-derived nanoparticles

Despite the promising findings of nanoparticle effects against trypanosomiasis, however, additional research will be needed to verify this expectation.

The general mechanism that has been suggested and explained to be responsible for protozoan death after treatment with nanoparticles has been illustrated in Fig. 3 and the recent updates against protozoan parasites using green nanoparticles were summarized in Table 1.

Conclusion

With the growing number of parasitic disease cases worldwide, it is critical to seek out novel and effective medications capable of curing these parasitic infections with little or no adverse effects. The usage of nanoparticles for the treatment of numerous parasite illnesses has recently expanded due to their unique structural properties. Recent research on green nano synthesis has revealed that it is a viable medicinal method. Testing these nanoparticles can cause DNA disruption, protein synthesis inhibition, membrane damage, damage to the ribosomes, ROS free radical generation and protozoal death. However, the mechanisms of NP antiplasmodial activity are not entirely understood or elucidated. Our understanding of protozoa parasites has significantly increased over the last few years. However, drug development to combat protozoan diseases is still urgently needed. This gap must be addressed more rigorously in future studies. However, the research suggests

additional *in vivo* and clinical studies are warranted to validate these findings as well as further work should be done for the identification of antiprotozoal activities of these nanoparticles under *in vivo* and *in vitro* models. Fig. 3 depicts the possible mechanisms of antiprotozoal activity of green-derived nanoparticles.

Author Contributions

All authors contributed to the design and the writing of the manuscript.

Conflicts of Interest

The authors declare no competing interests.

Data Availability

Not applicable.

Ethics Approvals

Not applicable.

Funding Source

The authors received no funding for this work.

References

Alajmi RA, WA Al-Megrin, D Metwally, H Al-Subaie, N Altamrah, AM Barakat, AE Abdel Moneim, TT Al-Otaibi, M El-Khadragy (2019). Anti-*Toxoplasma* activity of silver nanoparticles green synthesized with *Phoenix dactylifera* and *Ziziphus spina-christi* extracts which inhibits inflammation through liver regulation of cytokines in Balb/c mice. *Biosci Rep* 39:BSR20190379

Albalawi AE, AD Alanazi, MS Alyousif, A Sepahvand, K Ebrahimi, M Niazi, H Mahmoudvand (2021). The high potency of green synthesized copper nanoparticles to prevent the *Toxoplasma gondii* infection in mice. *Acta Parasitol* 66:1472–1479

Al-Salahy M, B Shnawa, G Abed, A Mandour, A Al-Ezzi (2016). Parasitaemia and its relation to hematological parameters and liver function among patients malaria in Abs, Hajjah, Northwest Yemen. *Interdiscip Perspect Infect Dis* 2016:5954394

Alti D, MV Rao, DN Rao, R Maurya, SK Kalangi (2020). Gold–silver bimetallic nanoparticles reduced with herbal leaf extracts induce ros-mediated death in both promastigote and amastigote stages of *Leishmania donovani*. *ACS Omega* 5:16238–16245

Aslam M, U Aslam, H Dur-e-Najaf (2022). Honey: A miraculous drug. *Agrobiol Records* 10:59–73

Avitabile E, N Senes, C D'avino, I Tsamesidis, A Pinna, S Medici, A Pantaleo (2020). The potential antimalarial efficacy of hemocompatible silver nanoparticles from Artemisia species against P. falciparum parasite. *PLoS One* 15:e0238532

Awad MA, EMA Olayan, MI Siddiqui, NM Merghani, SSAL Alsaif, AS Aloufi (2021). Antileishmanial effect of silver nanoparticles: Green synthesis, characterization, *in vivo* and *in vitro* assessment. *Biomed Pharmacotherapy* 137:111294

Azam SE, F Yasmeen, MS Rashid, MF Latif (2022). Physical factors affecting the antibacterial activity of Silver (Ag) and Zinc Oxide (ZnO) Nanoparticles (NPs), their application in edible and inedible food packaging and regulation in food products. *Intl J Agric Biosci* 11:181–193

Badirzadeh A, M Alipour, M Najm, A Vosoogh, M Vosoogh, H Samadian, AS Hashemi, ZJ Farsangi, SM Amini (2022). Potential therapeutic effects of curcumin coated silver nanoparticle in the treatment of cutaneous leishmaniasis due to *Leishmania major in-vitro* and in a murine model. *J Drug Deliv Sci Technol* 74:103576

Bahuguna A, DS Rawat (2020). An overview of new antitubercular drugs, drug candidates and their targets. *Med Res Rev* 40:263–292

Bajwa HUR, MK Khan, Z Abbas, R Riaz, TU Rehman, RZ Abbas, MT, Aleem, A Abbas, MM Almutairi, FA Alshammari, Y Alraey, A Alouffi (2022). Nanoparticles: Synthesis and their role as potential drug candidates for the treatment of parasitic diseases. *Life* 12:750

Belete TM (2020). Recent progress in the development of new antimalarial drugs with novel targets. *Drug Des Dev Ther* 14:3875–3889

Brito C, C Lourenço, J Magalhães, S Reis, M Borges (2023). Nanoparticles as a delivery system of antigens for the development of an effective vaccine against *Toxoplasma gondii*. *Vaccines* 11:733

Brito TK, RLS Viana, CJG Moreno, JDS Barbosa, FLDS Júnior, MJCD Medeiros, RF Melo-Silveira, J Almeida-Lima, DDL Pontes, MS Silva, HAO Rocha (2020). Synthesis of silver nanoparticle employing corn cob xylan as a reducing agent with anti-Trypanosoma cruzi activity. *Intl J Nanomed* 15:965–979

Chandrasekar BS, S Yadav, ES Victor, S Majumdar, M Deobagkar-Lele, N Wadhwa, S Podder, M Das, D Nandi (2015). Interferon-gamma and nitric oxide synthase 2 mediate the aggregation of resident adherent peritoneal exudate cells: Implications for the host response to pathogens. *PLoS One* 10:e0128301

Chatterjee AK, R Chakraborty, T Basu (2014). Mechanism of antibacterial activity of copper nanoparticles. *Nanotechnology* 25:135101

Chu KB, FS Quan (2021). Advances in *Toxoplasma gondii* vaccines: Current strategies and challenges for vaccine development. *Vaccines* 9:413

Costa IN, M Ribeiro, PS Franco, RJ da Silva, TE de Araújo, ICB Milián, LC Luz, PM Guirelli, G Nakazato, JR Mineo, TWP Mineo, BF Barbosa, EAV Ferro (2020). Biogenic silver nanoparticles can control *Toxoplasma gondii* infection in both human trophoblast cells and villous explants. *Front Microbiol* 11:623947

Degla LH, J Kuiseu, PA Olounlade, S Attindehou, MS Hounzangbe-Adote, PA Edorh, L Lagnika (2022). Use of medicinal plants as alternative for the control of intestinal parasitosis: Assessment and perspectives. *Agrobiol Rec* 7:449

Dunay IR, K Gajurel, R Dhakal, O Liesenfeld, JG Montoya (2018). Treatment of toxoplasmosis: Historical perspective, animal models, and current clinical practice. *Clin Microbiol Rev* 31:e00057-17

Dutta PP, M Bordoloi, K Gogoi, S Roy, B Narzary, DR Bhattacharyya, PK Mohapatra, B Mazumder (2017). Antimalarial silver and gold nanoparticles: Green synthesis, characterization and *in vitro* study. *Biomed Pharmacother* 91:567–580

Ebiloma GU, EO Balogun, EJ Cueto-Díaz, C Dardonville (2019). Alternative oxidase inhibitors: Mitochondrion-targeting as a strategy for new drugs against pathogenic parasites and fungi. *Med Res Rev* 39:1553–1602

Ezzi A, MBA Salahy, BH Shnawa, GH Abed, AM Mandour (2017). Changes in levels of antioxidant markers and status of some enzyme activities among falciparum malaria patients in Yemen. *J Microbiol Exp* 4:00131

Fernandes VS, RD Rosa, LA Zimmermann, KR Rogério, AE Kümmerle, LSC Bernardes, CS Graebin (2022). Antiprotozoal agents: How have they changed over a decade? *Arch Pharm* 355:e2100338

Galatage ST, AS Hebalkar, SV Dhobale, OR Mali, PS Kumbhar, SV Nikade, SG Killedar (2021). Silver nanoparticles: Properties, synthesis, characterization, applications and future trends. *In: Silver micro-nanoparticles-properties, synthesis, characterization and applications*, pp:61–80. IntechOpen, London

Gaona-López C, LK Vazquez-Jimenez, A Gonzalez-Gonzalez, T Delgado-Maldonado, E Ortiz-Pérez, B Nogueda-Torres, A Moreno-Rodríguez, K Vázquez, E Saavedra, G Rivera (2023). Advances in protozoan epigenetic targets and their inhibitors for the development of new potential drugs. *Pharmaceuticals* 16:543

Ghazali SZ, NRM Noor, KMF Mustaffa (2022). Anti-plasmodial activity of aqueous neem leaf extract mediated green synthesis-based silver nitrate nanoparticles. *Prepar Biochem Biotechnol* 52:99–107

Gujjari L, H Kalani, SK Pindiprolu, BP Arakareddy, G Yadagiri (2022). Current challenges and nanotechnology-based pharmaceutical strategies for the treatment and control of malaria. *Paras Epidemiol Contr* 17:e00244

Halonen S, FC Chiu, L Weiss (1998). Effect of cytokines on growth of *Toxoplasma gondii* in murine astrocytes. *Infect Immun* 66:4989–4993

Hano C, BH Abbasi (2021). Plant-based green synthesis of nanoparticles: Production, characterization and applications. *Biomolecules* 12:31

Hunter CA, LD Sibley (2012). Modulation of innate immunity by *Toxoplasma gondii* virulence effectors. *Nat Rev Microbiol* 10:766–778

Huq MA, M Ashrafudoulla, MM Rahman, SR Balusamy, S Akter (2022). Green synthesis and potential antibacterial applications of bioactive silver nanoparticles: A review. *Polymers* 14:742

Imarhiagbe O (2021). Aspects of the biology and ethnobotany of parasitic angiosperm species in Nigeria. *In: Parasitic Plants*. Gonzalez AM, H, Sato (Eds). IntechOpen, London

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

Khashan KS, GM Sulaiman, SA Hussain, TR Marzoog, MS Jabir (2020). Synthesis, characterization and evaluation of anti-bacterial, anti-parasitic and anti-cancer activities of aluminum-doped zinc oxide nanoparticles. *J Inorg Organometall Polym Mater* 30:3677–3693

Kiran H, S Kousar, F Ambreen, R Ilyas, S Abbas (2022). Effect of plant-based feed on the antioxidant enzymes, biochemical and hematological parameters of *Oreochromis niloticus*. *Contin Vet J* 2:67–75

Kumar SV, S Rajeshkumar (2018). *Plant-based synthesis of nanoparticles and their impact*. Nanomaterials in Plants, Algae and Microorganisms. Elsevier, Amsterdam, The Netherlands

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

Mohammadi L, K Pal, M Bilal, A Rahdar, G Fytianos, GZ Kyzas (2021a). Green nanoparticles to treat patients with Malaria disease: An overview. *J Mol Str* 1229:129857

Mohammadi M, L Zaki, A KarimiPourSaryazdi, P Tavakoli, A Tavajjohi, R Poursalehi, H Delavari, F Ghaffarifar (2021b). Efficacy of green synthesized silver nanoparticles *via* ginger rhizome extract against *Leishmania major in vitro*. *PLoS One* 16:0255571

Mahmoodi S, A Elmi, S Hallaj-Nezhadi (2018). Copper nanoparticles as antibacterial agents. *J Mol Pharm Org Proc Res* 6:1–7 140

Nawaz M, J Zhou, I Khalid, A Shamim, A Hussain, Z Ahmed, M Waqas, I Ahmed, MI Malik (2022). Antiparasitic activity of plants extract against gastrointestinal nematodes and *Rhipicephalus microplus*. *Intl J Vet Sci* 11:474–478

Patra S, S Mukherjee, AK Barui, A Ganguly, B Sreedhar, CR Patra (2015). Green synthesis, characterization of gold and silver nanoparticles and their potential application for cancer therapeutics. *Mater Sci Eng C* 53:298–309

Pritt B (2020). Common parasites. *Pathology* 52:49

Raghav A, SK Ansari, AK Singh, P Tripathi, S Agarwal, R Giri, SG Ali, HM Khan (2023). *Nanotechnology-Based Promising Therapeutic Approaches Against Protozoan*. Pathobiology of Parasitic Protozoa: Dynamics and Dimensions. Springer, The Nertherlands

Raj S, S Sasidharan, S Balaji, P Saudagar (2020). An overview of biochemically characterized drug targets in metabolic pathways of Leishmania parasite. *Parasitol Res* 119:2025–2037

Rani R, B Narsiman, RS Varma, R Kumar (2022). Gum-based nanocapsules comprising naphthoquinones enhance the apoptotic and trypanocidal activity against *Trypanosoma evansi*. *Eur J Pharm Sci* 171:106118

Rokkas T, JP Gisbert, P Malfertheiner, Y Niv, A Gasbarrini, M Leja, F Megraud, C O'Morain, DY Graham (2021). Comparative effectiveness of multiple different first-line treatment regimens for *Helicobacter pylori* Infection: A Network Meta-analysis. *Gastroenterology* 161:495–507

Saadatmand M, GRL Al-Awsi, AD Alanazi, A Sepahvand, M Shakibaie, S Shojaee, R Mohammadi, H Mahmoudvand (2021). Green synthesis of zinc nanoparticles using *Lavandula angustifolia* Vera. extract by microwave method and its prophylactic effects on *Toxoplasma gondii* infection. *Saud J Biol Sci* 28:6454–6460

Shani WS, B Shnawa, N Waheda (2012). Levels of Immunoglobulins and complements in sera of patients with toxoplasmosis. *Basrah J Sci B* 30:72–77

Shani WS, BH Shnawa, N Elia (2010). Detection of phagocytic activity and T, B and T–Helper lymphocytes in peripheral blood of women infected with toxoplasmosis in Basrah province. *Al-Qadisiya J Sci* 15:18–25

Shibeshi MA, ZD Kifle, SA Atnafie (2020). Antimalarial drug resistance and novel targets for antimalarial drug discovery. *Infect Drug Resist* 13:4047–4060

Shnawa BH (2017). Molecular diagnosis of *Entamoeba histolytica*, *Entamoeba dispar* and *Entamoeba moshkovskii*: An update review. *Annu Res Rev Biol* 21:1–12

Shnawa BH (1995). Biological and lmmunological studies on *Giardia lamblia. Ph. D. Thesis*, University of Basrah, Basrah, Iraq

Shnawa BH, PJ Jalil, PK Aspoukeh, DA Mohammed, DM Biro (2022a). Protoscolicidal and biocompatibility properties of biologically fabricated zinc oxide nanoparticles using *Ziziphus spina-christi* leaves. *Pak Vet J* 42:517–525

Shnawa BH, PJ Jalil, SM Hamad, MH Ahmed (2022b). Antioxidant, protoscolicidal, hemocompatibility, and antibacterial activity of nickel oxide nanoparticles synthesized by *Ziziphus spina-christi*. *BioNanoSci* 12:1264–1278

Shnawa BH, SJ Al-Ali, SO Swar (2021). Nanoparticles as a new approach for treating hydatid cyst disease. *Vet Pathobiol Publ Health* 1:180–185

Shnawa BH, S Gorony, KM Khalid (2017). Efficacy of *Cyperus rotundus* rhizomes-tubers extracts against protoscoleces of *Echinococcus granulosus*. *World J Pharm Res* 6:157–179

Sjöholm E, N Sandler (2019). Additive manufacturing of personalized orodispersible warfarin films. *Intl J Pharm* 564:117–123

Snoussi Y, I Sifaoui, ME Garah, AM Khalil, JE Barroso, M Jouini, S Ammar, J Lorenzo-Morales, MM Chehimi (2022). Green, zero-waste pathway to fabricate supported nanocatalysts and anti-kinetoplastid agents from sugarcane bagasse. *Waste Manage* 155:179–191

Sobhy H, TR AboElnaga T, TS Behour, EA Razin (2021). *In vitro* trypanocidal activity of essential oils of some plants against *Trypanosoma evansi*. *Intl J Vet Sci* 10:191–195.

Souza AO, JWDF Oliveira, CJG Moreno, MJCD Medeiros, MM Fernandes-Negreiros, FRM Souza, DL Pontes, MS Silva, HAO Rocha (2022). Silver nanoparticles containing fucoidan synthesized by green method have anti-*Trypanosoma cruzi* activity. *Nanomaterials* 12:2059–2077

Stevens AJ, R Abraham, KA Young, CC Russell, SN McCluskey, JR Baker, B Rusdi, SW Page, R O'Handley, M O'Dea, S Abraham, A McCluskey (2022). Antigiardial activity of novel guanidine compounds. *ChemMedChem* 17: e202200341

Swar SO, BH Shnawa (2020). Ultrastructural and molecular characterization of *Sarcocystis* species derived from macroscopic sarcocysts of domestic sheep and goats in Soran city, Erbil, Iraq. *World Vet J* 10:540–550

Syed Z, K Sonu, A Dongre, G Sharma, M Sogani (2020). A review on Hydroxamic acids: Widespectrum chemotherapeutic agents. *Intl J Biol Biomed* 14:75–88

Tabassum S, M Arshad, S Naz, D Danabas, RA Bhat (2022). Prevalence of protozoan parasites in gastrointestinal tract of goats. *Contin Vet J* 2:93–102

Thakare R, A Dasgupta, S Chopra (2021). Update on nifurtimox for treatment of chagas disease. *Drugs Today* 57:251–263

Vergara-Duque D, L Cifuentes-Yepes, T Hincapie-Riaño, F Clavijo-Acosta, G Juez-Castillo, B Valencia-Vidal (2020). Effect of Silver Nanoparticles on the Morphology of *Toxoplasma gondii* and Salmonella braenderup. *J Nanotechnol* 2020:9483428

Wang X, D Zhang, N Jiang, X Wang, N Zhang, K Zhang, X Sang, Y Feng, R Chen, N Yang, O Chen (2021). Induction of apoptosis in *Trypanosoma brucei* following endocytosis of ultra-small noble metal nanoclusters. *Nano Today* 38:101122.

White N, S Pukrittayakamee, T Hien, M Faiz, O Mokuolu, A Dondorp (2014). Erratum: Malaria *Lancet* 383:723–735

WHO - World Health Organization (2022a). Leishmaniasis. Available at: https://www.who.int/news-room/fact-sheets/detail/leishmaniasis (Accessed: June 1 2022)

WHO - World Health Organization (2022b). Chagas disease [(Accessed: 14 April 2022)]. Available online: https://www.who.int/health-topics/chagas-disease#tab=tab_1 [Ref list].2022 b.

Xu L, YY Wang, J Huang, CY Chen, ZX Wang, H Xie (2020). Silver nanoparticles: Synthesis, medical applications and biosafety. *Theranostics* 10:8996–9031

Zaheer T, M Imran, K Pal, MS Sajid, RZ Abbas, AI Aqib, MA Hanif, SR Khan, MK Khan, SU Rahman (2021). Synthesis, characterization and acaricidal activity of green-mediated ZnO nanoparticles against Hyalomma ticks. *J Mol Str* 1227:129652

Zein R, I Alghoraibi, C Soukkarieh, A Alahmad (2022). Investigation of cytotoxicity of biosynthesized colloidal nanosilver against local *Leishmania tropica*: *In vitro* Study. *Materials* 15:4880