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Article

Green Synthesized Nanoparticles as a Novel Alternative Paradigm for the Therapeutic Management of Intracellular Protozoa

Raghda Mahmood Hamad[1], Mohammed Mohsen Sharqat Ali Al-Jumaili[2]

1. Department of Science , College of Basic Education _Shirqat, Tikrit University, Iraq.
2. Ministry of Education Directorate General of Salahuddin Education,Iraq
*Correspondence: raghada.hamad21@tu.edu.iq[1] , aljamilimohammed5@gmail.com[2]

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Abstract: Intracellular parasitic protozoa such as Toxoplasma gondii, Leishmania spp., and Plasmodium falciparum continue to be a huge global public health and socio-economic problem. Modern frontline chemotherapy [remains] largely dependent on drugs with a history of development decades ago, including pyrimethamine, sulfadiazine, and antimonials. However, alarming rates of drug resistance, extreme systemic toxicities, and poor bioavailability have increasingly compromised these standard regimens, in addition to their inability to safely eradicate latent encysted tissue stages. Applied parasitology has been used for many years however, more recently the interface between green nanotechnology and applied parasitology has presented a novel, innovative alternative. This update review elaborates current advances during the past five years (2022–2026) on the use of biogenic metal and metal oxide nanoparticles (NPs), in particular Ag, Au, Se, ZnO and CuO mediated with various bioactive plant extracts as innovative new anti parasitic platforms. We present a rigorous assessment of their preclinical biomedicinal efficacy against both tachyzoite and bradyzoite stages, elucidate their multi-targeted intracellular mechanisms of action (ROS mediated apoptosis, membrane disruption, downregulation of key virulence genes) and identify current physiological bottlenecks that may limit clinical translation. Finally, we discuss bioengineering strategies that will be required to design optimized, targeted, and non-toxic nanoscale molecular formulations for biomedical applications in the future

Keywords: Green-synthesized nanoparticles; Antiparasitic nanomedicine; Drug resistance; Intracellular protozoa; Plant-mediated nanotechnology.

Introduction

Some of the most resilient and evolutionarily fit human pathogens belong to the family of intracellular protozoan parasites. Of these, Toxoplasma gondii is by far the most widespread parasite of vertebrates capable of infecting almost all endothermic species; approximately one third of the human population [1]. Infections in immunocompetent hosts are often subclinical or asymptomatic due

to a strong cell-mediated immune response, but acute toxoplasmosis is a serious, life-threatening danger in populations that are not immunocompetent.

In pregnant women, primary infection can lead to congenital transmission, stillbirth, resulting in spontaneous abortion, or severe neonatal neurological and ocular abnormalities such as hydrocephalus, microcephaly, and chorioretinitis [2]. In immunocompromised patients, such as those undergoing chemotherapy, organ transplant recipients, or individuals with HIV/AIDS, the reactivation of latent tissue cysts routinely causes catastrophic necrotic encephalitis and systemic dissemination [3]. The clinical controlling of toxoplasmosis has remained stagnant for over half a century, relying primarily on the synergistic combination of pyrimethamine and sulfadiazine. This standard regimen acts by inhibiting the folate synthesis pathway of the parasite, effectively suppressing tachyzoite proliferation during the acute phase of infection. However, the therapeutic utility of this gold standard is critically undermined by severe, dose-limiting bone marrow suppression, leukopenia, thrombocytopenia, and frequent allergic hypersensitivity reactions [2].

More egregiously, these conventional chemical drugs lack the pharmacokinetic capacity to penetrate the dense, chitinous walls of latent tissue cysts (bradyzoites) situated within the central nervous system and muscular tissues, making complete radical cure unattainable and leaving the host highly susceptible to lifelong chronic recurrence [4].

Clinical challenges associated with this marked rise in cases are especially exacerbated by the rapid emergence of drug-resistant strains of these parasites, facilitated by prolonged maintenance therapy and sub-therapeutic dosing schedules at the global level. As a result, there is a critical and irrefutable academic necessity to design new antiprotozoal agents in which enhanced biocompatibility, improved targeted drug delivery, and new multi-valent mechanisms of action which can clear both active and encysted forms with minimal host toxicity. [5].

2. The Pattern of Green Nanotechnology in Medical Parasitology

Nanotechnology has basically revolutionized modern drug discovery by introducing materials engineered at the nanoscale (1–100 nm), which possess unique physicochemical properties, including an extraordinarily high surface-area-to-volume ratio, improved reactivity, and the ability to cross formidable biological barriers [6]. Though, conventional chemical and physical methods of nanoparticle synthesis commonly involve the utilization of hazardous reducing agents, toxic organic solvents, and high energy inputs, which inevitably lead to environmental contamination and the preservation of toxic chemical residues on the nanoparticle surface, rendering them unsuitable for delicate in vivo biomedical applications [7].

To avoid these limitations, green synthesis protocols have been pioneered as an eco-friendly, cost-effective, and highly biocompatible alternative [8]. Green synthesis utilizes non-toxic biological systems principally medicinal plant extracts, but also fungi, bacterial cultures, and intracellular enzymes to serve as both reducing and capping agents that convert metallic salts into stable, functionalized nanostructures [9].

Medicinal plants are particularly favored due to their rich reservoir of secondary metabolites, including polyphenols, flavonoids, terpenoids, alkaloids, , and saponins [8]. During biogenic synthesis, these complex phytochemicals actively donate electrons to reduce metal ions (e.g., Ag^+, Zn^{2+}) into zero-valent nanoparticles, while simultaneously forming an outer stabilizing layer (capping) around the newly formed core. This normal corona prevents nanoparticle agglomeration and imparts innate synergistic anti parasitic properties to the nanomaterial, enhancing its overall therapeutic index while maintaining low cytotoxicity toward host mammalian tissues [7].

3. Evaluation of Diverse Biogenic Metallic and Oxide Nanoparticles

A wide array of metal and metal oxide precursors have been subjected to biogenic synthesis and screened for antiparasitic efficacy, each revealing distinct structural characteristics and biological interactions.

a. Phyto-mediated Silver Nanoparticles (AgNPs)

Even though there is a whole range of metallic nanomaterials with reported antiparasitic activity, silver nanoparticles obtained by green protocols has shown the most pronounced and

universal action against intracellular protozoa. For example, biogenic AgNPs produced using extracts from the pharmacologically active plants Ziziphus oxyphylla, Mentha piperita or Phoenix dactylifera exhibit a strong in vitro tachyzoiticidal and in vivo scolicidal activity [10]. This natural capping layer can make sure that the silver core can release silver ions (Ag+) to biological infection in a controlled and sustained way. New peer-reviewed evaluations show that green AgNPs can reduce acute parasitic burdens of murine models by ~ 85% within the dosages of green AgNPs at a much lower dosage than the standard cytotoxic levels of synthetic pyrimethamine. The remarkable efficacy of our nanoparticles is attributed to their selective targeting of rapidly dividing parasitic cells at the expense of healthy host macrophages and splenocytes [3].

b. Biogenic Zinc Oxide Nanoparticles (ZnO-NPs)

Green synthesized zinc oxide nanoparticles have gained significant scientific attention because they possess high chemical stability, low cost, and good safety despite being one of the most effective biocides utilized in agriculture [10]. The ZnO-NPs works mainly by creating a lot of mechanical and structural damage on the external pellicle of the parasite. Mice infected with virulent T. gondii strains that had subsequently been treated with plant-mediated ZnO-NPs in vivo comparative trials performed in 2025 and exhibited a remarkable parasite clearance in hepatic and splenic tissues. Concomitant with this clearance was a significant upregulation of host protective cytokines and a significant increase in overall post infection survival, making ZnO-NPs a promising replacement for toxic sulfonamides [10].

c. Green Gold Nanoparticles (AuNPs) and Novel Challengers

Gold nanoparticles prepared via biogenic synthesis provide unparalleled biocompatibility and single optical properties that facilitate trackable drug delivery. Although historically constrained by high precursor material costs, green AuNPs excel in their pharmacokinetic ability to cross tightly regulated biological boundaries, most notably the blood brain barrier (BBB), making them major candidates for treating neurological parasitic infections [10].

Simultaneously, novel nanomaterials like biogenic selenium (Se-NPs) and copper oxide (CuO-NPs) are being pioneered. Selenium nanoparticles synthesized via probiotic bacterial strains have shown a unique capability to stimulate the host's innate immune response, triggering enhanced macrophage phagocytosis and nitric oxide production, which actively helps in the intracellular allowance of protozoan invaders [4].

4. Comprehensive Intracellular Mechanisms of Action

The superior efficacy of green synthesized nanoparticles over conventional monotherapy lies in their multi-targeted, simultaneous modes of action, which exert catastrophic physiological stress upon the parasite through separate chemical and physical pathways [10].

a. Induction of Oxidative Stress and Mitochondrial Dysfunction

The baseline chemical mechanism driving biogenic nanoparticle lethality is the continuous, uncontrolled induction of Reactive Oxygen Species (ROS), including hydroxyl radicals, superoxide anions, and hydrogen peroxide within the parasite's cytoplasm. Intracellular protozoa are extraordinarily sensitive to oxidative variations due to their rudimentary antioxidant defense systems [11].

Upon entry, metallic nanoparticles exchange with cytoplasm enzymes and produce large amounts of ROS, exceeding the internal homeostasis of the parasite. Such sudden oxidative stress leads to the lipid peroxidation of mitochondrial membrane producing the complete collapse of mitochondrial membrane potential. When deprived of adenosine triphosphate (ATP) synthesis, the parasite experiences a catastrophic sequence of cellular breakdown, including chromatin condensation and DNA fragmentation, similar to the canonical mechanisms of metazoan programmed cell death, or apoptosis. [12].

a. The deliberate rupture of the pellicle and cytoplasmic exudation

At the same time, nanoparticles create physical trauma to the parasite's outer layer. Formed of the plasma membrane and an underlying inner membrane complex, the complex pellicle that surrounds T. gondii is critical for shape, osmotic stability and motility. Green NPs readily adsorb to the surface glycoproteins of the pellicle owing to opposite electrostatic charge and high surface energy. This binding gives rise to localized mechanical perforation, erosion and pitting of the membrane. Scilab: Ultra-structural assessments through Scanning Electron Microscopy (SEM) commonly account severe morphological deformations, embracing the detachment of the permissible crescent shape, education swelling, as well as deep membrane embeddings.

This physical disintegration eventually brings a complete loss of osmotic regulation, which triggers the leak of important intracellular proteins, ions and factors into the extracellular matrix, causing an immediate cytolysis [10].

b. Downregulation of virulence-related secreted factors

In addition to physical and oxidative damage, molecular assays suggest that biogenic nanoparticles can influence genetic machinery involved in parasitic infectivity and infectivity of host cells. T. gondii intracellular invasion is a highly regulated and active process initiated through the consecutive secretion of proteins from specialized apical organelles including micronemes (MICs), rhoptries (ROPs) and dense granules (GRAs) [11].

Exposure to plant-mediated nanoparticles markedly inhibits the transcription and translation of essential virulence genes, including MIC1, MIC3, ROP16, and ROP18 [11].

These important target molecules are prepared to be blinded by nanoparticles, where this blinding prevents contact with host cells, neutralizes attachment mechanics, and impairs the establishment of the parasitophorous vacuole for intracellular survival and replication [13].

5. Comparative Synthesis Matrix of Recent Nanomedicinal Studies

In order to systematically overview the field, the table below cross compares different green synthesized nanostructures tested on protozoan models with a timeline of 2022–2026, including the biological source, precise mode of action, and recognised development limitations.

Table 1. -Comparison of different green synthesized nanosstructures characterized using protozoan model.

Nanomaterial Core	Biogenic / Botanical Source	In Vitro & In Vivo Antiparasitic Efficacy	Identified Developmental Bottleneck
Silver (AgNPs)	Phoenix dactylifera (Date Palm)	Exhibits 85% reduction in acute tachyzoite load in vivo; completely destroys free extracellular parasites.	High potential for long-term bioaccumulation in maternal liver and kidneys.
Zinc Oxide (ZnO)	Camellia sinensis (Green Tea)	Causes total collapse of pellicle osmotic integrity; induces high levels of localized intracellular ROS.	Maximum antiprotozoal efficacy requires direct UV/visible light photo-activation.
Gold (AuNPs)	Zingiber officinale (Ginger)	Demonstrates excellent systemic safety indices; efficiently crosses the BBB to reach cerebral tissues.	Prohibitive economic costs associated with noble metal chemical precursors.
Selenium (Se-NPs)	Lactobacillus crispatus (Biogenic)	Actively upregulates host macrophage phagocytic activity and	Exhibits narrow therapeutic windows; slight overdosage

		systemic nitric oxide generation.	induces host hepatotoxicity.
Copper Oxide (CuO)	Azadirachta indica (Neem)	Strong inhibition of enzymatic replication pathways; disrupts cellular division cycles.	Displays moderate baseline toxicity toward human healthy fibroblastic cell lines.

6. Physiological Bottlenecks and Barriers to Clinical Translation

Although the doubtable pharmacological benefits exhibited by biogenic nanomaterials in preclinical conditions, within clinic to laboratory transfers, several technical and biological requirements may challenge the clinical application of those agents.

❖ **Long-lasting Pharmacokinetics and Tissue Bioaccumulation**

The major safety issue related to metallic nanoparticles is that there is a lack of sufficient and integrated multi-generational in vivo pharmacokinetic and biodistribution data to assess their safety. Short-term toxicity screens rarely report positive tolerability data, and heavy metal cores—especially silver and copper—are notoriously rugged against rapid physiological degradation. After systemic administration, a large fraction of these particles become deposed in the reticuloendothelial system, mainly in the liver, spleen and renal cortex. Aquatic monomeric silver has potential to sequester in organelle and tissues As metalloestrogens such process are yet not understood for chronic low-grade nephropathy and systemic argyria [3].

❖ **The Difficulty of Getting into the Brain and Penetrating the Tissue Cysts**

For chronic parasitic infections such as toxoplasmosis, the latent tissue cyst (bradyzoite) represents the main therapeutic target and is, by nature of its limited host cell exit, mostly inaccessible (sequestered) within the brain tissue of chronic host animals. Making it almost impossible to kill these cyst since any drug candidate must initially go over the extremely selective Blood-Brain Barrier (BBB) [14].

Although some green nanoparticles, especially gold nanostructures, have surface features that enable only slight passive diffusion via the BBB, the majority of metallic nanoparticles are rapidly recognized and removed by circulating opsonins and macro-phagocytic cells before entering the central nervous system [15].

The impermeable physical barrier produced by the densely-walled, chit nous structural "wall" surrounding mature bradyzoite tissue cysts drastically limits nanoparticle entry and protects the dormant parasites from complete biological eradication [13].

❖ **Standardization of manufacture and quality control**

From a industrial manufacturing point of view, green synthesis protocols are plagued by natural biological reproducibility issues. The plant phytochemical content of a botanical extract is inherently shaped by pre-, during and post-harvest external factors, such as where the plant was grown (geographic location) [15]. when it was grown (seasonal climate), when it was harvested, and what soil it was grown in.

Thus, these variable plant extracts yield major inconsistencies in terms of the size distributions, surface charge, and capping efficiencies among different batches of produced nanoparticles [13].

However, various factors render commercial scale-up very challenging, especially due to the fact that the global regulatory landscapes will not allow initiation of human clinical trials (and hence delivery of curative medicinal products) without stringent structural homogeneity and reproducible pharmacological profiles [7].

.7. Strategic Future Directions and Bioengineering Horizons

In the long run, the physiological limitations must be overcome to achieve the full potential of biogenic nanotechnology through future research moving away from generic empirical screening to tailored surface engineering and multi-functional nano-architecture.

- **Later Designs**: The later designs can concentrate more on surface functionalization with selective penetration based on the anchoring of specific CPPs or ligand molecules like transferrin or lactoferrin on to the green capping corona. This will be done using a modification that will take

advantage of receptor the mediated transcytosis pathways, actively pushing the nanoparticles through the Blood-Brain Barrier to directly access the cerebral parasitic niches [16].

- **Development of Polymeric and Hybrid Nanoemulsions:** The move from simple solid metal cores to polymer-coated green nanoemulsions or lipid-based hybrid nanocarriers would have the two-fold benefits. The bioinert polymer protects the core from premature opsonization early after systemic administration, favors its passage through the thick chitinous walls of tissue cysts and allows the controlled release of the drug on target cells.
- However, future protocols shall be executed through combination of state-of-the-art high-performance liquid chromatography (HPLC) with mass spectrometry to longitudinally profile, separate and characterize specific phytochemical fractions involved in metal reduction. Researchers can reach excellent structural homogeneity and reproducible manufacturing profiles [17]. shifting from crude whole-plant extracts to highly quantified, pure molecular fractions.

Conclusion

Green nanotechnology is now at the forefront of drug discovery and integrated into applied medical parasitology, representing a very efficient, eco-friendly and mechanical revolution. Biogenic nanoparticles fabricated along plant processes also overcome strict therapeutic restrictions common in conventional single-molecule treatment since they kill intracellular protozoa through a spectrum of chemical and physical disruption antics, thus making the establishment of genetic resistance in the parasite nearly impossible. A clearer picture of long-term bioaccumulation dynamics in human tissues needs to be created and smart, surface-engineered nanomaterials designed as vehicles capable of penetrating the cyst wall to gain access to cyst contents need to be elucidated: all these represent areas requiring urgent, coordinated efforts to best exploit the potential of existing nanomaterials from lab models to validated human medicines..

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