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Emerging Therapeutic Strategies Against *Entamoeba Histolytica*: Thioredoxin Reductase Inhibition and Nanoparticle-Based Drug Delivery

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Abstract

Amoebiasis caused by *Entamoeba histolytica* is still a clinically important parasitic disease, both intestinal and extraintestinal in nature, with endemic areas being limited by sanitation and access to molecular diagnosis and treatment especially at lower income countries. Metronidazole and related nitroimidazoles remain essential for invasive disease, their shortcomings include adverse effects, limited activity against luminal cysts, and requirements for a second luminal agent and reduced susceptibility.

This narrative review highlights the new evidence on novel antiamoebic strategies, focusing on metronidazole resistance, thiol-based redox metabolism, potential drug targets such as thioredoxin reductase, auranofin and related inhibitors, as well as nanoparticle-based delivery.

Using pre-defined search terms and inclusion/exclusion criteria, a literature review was conducted based on available PubMed, Scopus, Web of Science and Google Scholar using search methodology. Thioredoxin reductase is the most strongly target-based evidence, due to absence of a classical glutathione system in *E. histolytica* and reliance on thioredoxin-linked pathways for oxidative-stress adaptation. Auranofin provides proof of concept for redox-directed therapy, although resistance adaption toxicity, and in vivo efficacy require additional clarification.

Although work with *E. histolytica* showed few promising studies supporting nanoparticles, data remains limited in other protozoa and should be regarded as more so supportive than conclusive. Future work should focus on validated models of *E. histolytica*, host-cell toxicity testing, pharmacokinetic profiling, and in vivo studies prior to clinical translation.

Keywords: *Entamoeba Histolytica*, Amoebiasis, Thioredoxin Reductase, Metronidazole Resistance, Nanoparticles.

AIMCJ. (2026),3; (2): 22-33
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Introduction

Entamoeba histolytica is an invasive intestinal protozoan parasite that causes amoebic colitis and extraintestinal disease, especially amoebic liver abscess (1-4).

Ingestion of the mature cysts via contaminated food or water leads to infection as these excyst in the upper intestine, followed by colonization and mucosal invasion in a small percentage of cases (2, 4). Despite widespread use of microscopy, morphologically similar Entamoeba species confound microscopic diagnosis and therefore antigen detection and molecular assays may be a more accurate approach (5-7).

Current treatment is predominantly metronidazole or other nitroimidazoles for invasive disease, followed by a luminal agent to eradicate cyst carriage (8). This is a very useful therapeutic approach for many patients, but it does have certain limitations being associated with side effects, drug-drug interactions, incomplete activity against cyst stages and concerns of altered activation mechanisms leading to reduced susceptibility or induction of antioxidant responses (9-13).

These constraints warrant exploring therapeutic options that target more parasite-specific pathways, rather than solely targeting nitro-imidazole mechanisms.

One of the most attractive targets is thioredoxin reductase, a key enzyme in the thiol-driven redox system of *E. histolytica* (14-18). The parasite neither possesses the classical glutathione /glutathione reductase antioxidant system found in mammalian cells nor do they use it extensively during invasion of the host but utilizes thioredoxin-linked mechanisms to escape oxidative and nitrosative stress (14, 15). This dependency grounds a therapeutically exploitable weakness. Additionally, nanotechnology-mediated delivery system might offer advantages such as solubility, stability, bioavailability and parasite-directed delivery of antiamoebic agents (19, 20). The life cycle of *E. histolytica* is shown in Figure 1. This diagram illustrates the different stages of infection, such as invasion into the tissue, diagnosis and transmission through other sources in the environment. Therefore, the present review aims to provide a critical summary of novel developments regarding metronidazole resistance and redox-targeted therapy, as well as highlight important studies that investigate auranofin- and nanoparticle-based approaches against *E. histolytica* with mechanistic insight into their action. This review aims to systematically assess novel antiamoebic approaches beyond standard nitroimidazole therapy, focusing on the four following objectives:

(i) summarize mechanisms of metronidazole action and resistance-related adaptation;
(ii) evaluate thioredoxin reductase as a selective redox target;
(iii) summarize evidence for redox-directed inhibitors including auranofin and related compounds; and
(iv) critically discuss nanoparticle-based delivery methods and current evidence base in *E. histolytica.*

AIMCJ. (2026),3; (2): 22-33
doi.org/10.58564/AIMCJ3.2.2026.281

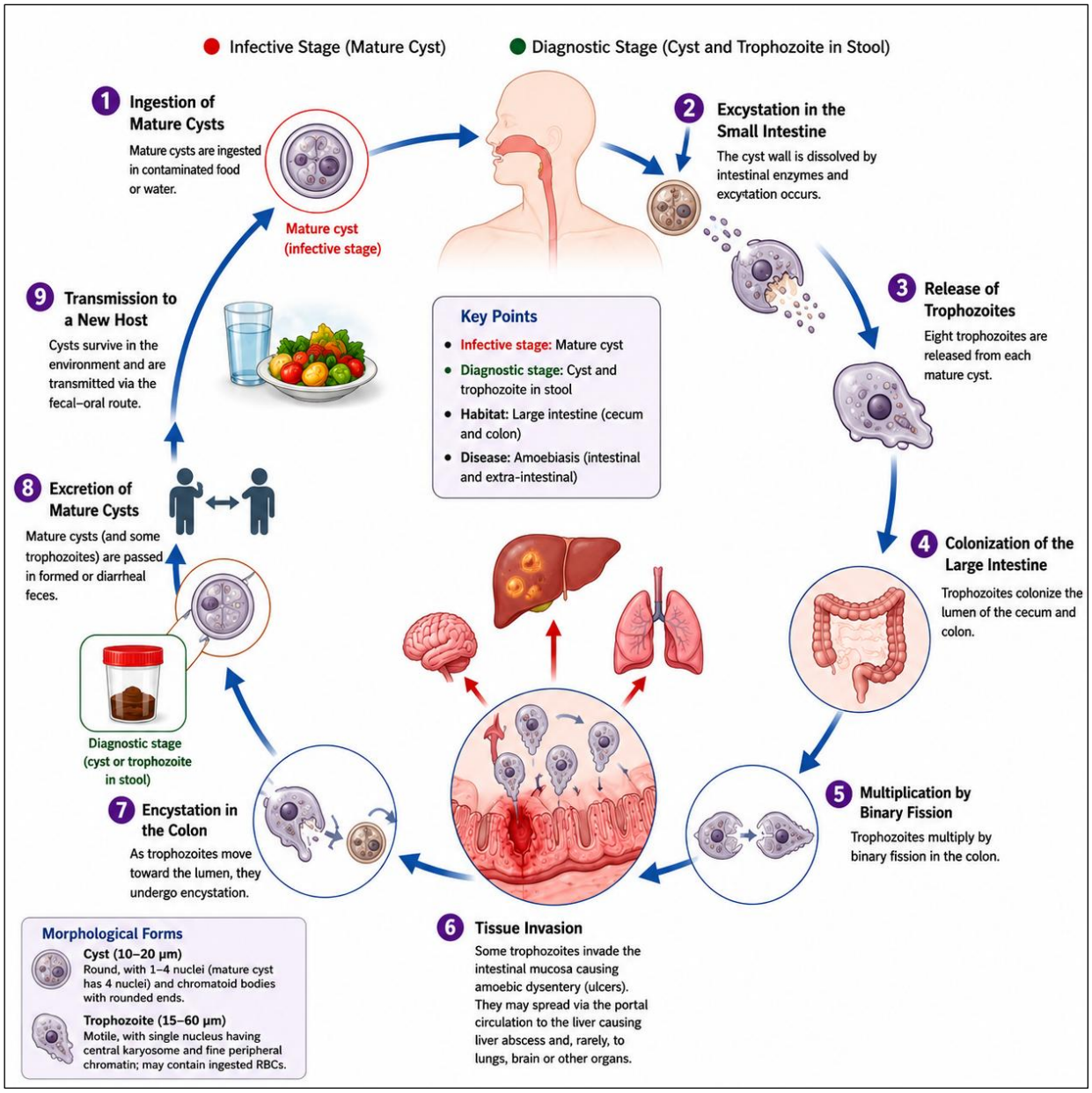

Figure 1: Schematic representation of the life cycle and pathogenic progression of *E. histolytica.* The infection begins after ingestion of mature cysts through contaminated food or water, excystation in small intestine, release and multiplication of trophozoites in the large intestine, tissue invasion causing intestinal and extraintestinal amoebiasis, encystation in the colon, and excretion of mature cysts in the feces for transmission to a new host. The mature cyst represents the infective stage while cysts and trophozoites detected in the stool constitute the diagnostic stage. This diagram was redrawn by the authors by using BioRender.com, based on the established life cycle of *E. histolytica* described in the literature (3, 21).

AIMCJ. (2026),3; (2): 22-33
doi.org/10.58564/AIMCJ3.2.2026.281

Material and Methods:

Study design

This narrative review summarizes the current evidence of novel therapeutics against *E. histolytica* with significant emphasis on metronidazole resistance, thioredoxin reductase, auranofin, and nanoparticle-based drug delivery strategies.

Search Strategy

A literature search on PubMed/MEDLINE, Scopus, Web of Science, and Google Scholar databases was conducted. Articles published between 1995 and 2026 were primarily included, with older foundational papers being included if they described core mechanisms or established principles in therapeutic design. By searching through the PubMed database, using various search terms such as *E. histolytica*, amoebiasis, metronidazole resistance, nitroimidazole, thioredoxin reductase, thiol redox metabolism/oxidative stress/auranofin nanoparticles/silver nanoparticles/copper oxide nanoparticles/nanocarriers, and antiamoebic therapy.

Inclusion criteria

Studies were included if they provided peer-reviewed English language articles that directly addressed one or more of the following themes: epidemiology or diagnosis relevant to treatment of amoebiasis, mechanisms of action and/or resistance toward metronidazole, redox metabolism in *E. histolytica*, thioredoxin reductase as a drug target, auranofin or related inhibitors delivered through different means (e.g., gold nanoparticles), synthetic or natural antiamoebic compounds pertinent for therapeutic development, nanoparticle-based antiparasitic approaches for amoebiasis therapy. Novel reviews with emphasis on summarizing recent developments focused on therapeutics against *E. histolytica*.

Exclusion criteria

We excluded articles if not amoebiasis or protozoal therapy-related; lacked sufficient methodological detail; were conference abstracts without available full text; were on non-biomedical nanoparticle applications only; discussed amoebae but were unrelated to drug development, parasite biology, or therapeutic targeting. We excluded duplicates and out of scope articles, such as ones with no full text available.

Study selection and data extraction

Initial screening was done on titles and abstracts, after which all relevant articles were subjected to full-text evaluation. Studies were extracted narratively rather than meta-analytically (due to heterogeneity in study designs, parasite stages, assays, compounds, and nanoparticle systems). Information that extracted included study type, target or intervention, experimental system, primary findings and highlights, relevance to antiamoebic therapy, and major limitations. We categorized the relevant evidence by thematic area and summarized it in Table 1.

Results:

Summary of selected evidence

The putative classifications identified from the review of literature were compiled schematically and serve four evidence categories for those putting forward new antiamoebic strategies: current nitroimidazole therapy limitations; metronidazole resistance and redox adaptation, thioredoxin reductase inhibition, and nanoparticle delivery or direct toxicity with nanoparticles. The most important evidence areas that may ultimately serve as targets in therapeutic development are summarized in Table 1.

AIMCJ. (2026),3; (2): 22-33
doi.org/10.58564/AIMCJ3.2.2026.281

Table 1: Highlighted evidence on potential novel therapeutic scenarios against *E. histolytica*.

Evidence area	Representative evidence	Key findings	Therapeutic relevance
Current treatment	WHO guidance: Coherence review: clinical review (11, 12)	Nitroimidazoles treat invasive trophozoites, but luminal agents needed for cyst clearance.	Combination therapy is still required; new strategies should inhibit both invasive disease and transmission
Limitations of Metronidazole	Pharmacology and toxicity reviews (13, 22, 23)	Adverse effects, interactions, intolerance, and rare neurotoxicity affect compliance	Safer alternatives or lower-dose combination strategies are needed
Mechanisms of resistance	Studies of susceptibility in vivo and in clinical trials (22, 24, 25)	Failure to activate drugs, altered antioxidant enzymes associated with reduced susceptibility	Instead of just assessing bacterial nim genes, resistance surveillance needs to use redox adaptation to the parasite
Redox biology	Involvement of thiol studies (14-17)	*E. histolytica* uses a combination of thioredoxin, thioredoxin reductase, and peroxiredoxin since the classic glutathione pathways are limited.	Metabolism of thiol compound as parasite target which can be related to drug.
Thioredoxin reductase	Functional and structural studies (16-18, 26)	EhTrxR supports NADPH-dependent redox balance − distinct from mammalian systems.	Another strategy is selective inhibitors, which could impair parasite viability.
Auranofin	Drug screening and structural work (26-28)	Redox-linked pathways, parasite virulence, and stress response are targets of Auranofin.	Redox-targeted therapy repurposing shows proof of concept but needs evaluation for safety and resistance.
Novel compounds	Natural and synthetic antiamoebic compounds (29-32)	Flavonoids, hydrazones, and related compounds display experimental antiamoebic activity.	Lead optimization and selectivity for the target are needed prior to translational use.
Nanoparticles	Nanomedicine and protozoal studies (19, 20, 33-39)	Nanoparticles with metallic and carrier functions can destroy parasites, lead to oxidative stress or facilitate drug release.	Nanocarriers may allow enhanced solubility as well as targeted and controlled release of redox-targeted drugs.
Safety gaps	Nanotoxicity and characterization reviews (19, 20, 40)	The safety and reproducibility of nanoparticles are influenced by characteristics such as particle size, morphology, surface charge, coating, dose, route of exposure, stability, and biological distribution. Nanoparticles that are poorly characterized may exhibit variable efficacy or even host-cell toxicity.	A clinical translation of nanoparticle-based antiamoebic therapy will require the delivery of strict standardized physicochemical characterization, host-cell cytotoxicity testing, in vivo safety assessment, and reproducibility assessments.

Biological and clinical context

The current literature indicates that *E. histolytica* pathogenesis mostly depends on a combination of parasite and host factors: trophozoite adhesion, contact/cytotoxicity, cysteine proteases, Gal/GalNAc lectin, amoebapores and invasion into tissues are all the crucial factors of intestinal and extraintestinal disease (3). The most relevant biological characteristics from a

AIMCJ. (2026),3; (2): 22-33
doi.org/10.58564/AIMCJ3.2.2026.281

therapeutic viewpoint, however, are the cyst stage of infectivity and transmission; trophozoite stage responsible for invasive disease; and parasite metabolic pathways necessary for survival in the intestine and tissue pathological stress. In working with preclinical models, the retained life-cycle diagram highlights why optimal therapy needs to not only suppress invasive trophozoites, but also prevent luminal persistence and environmental transmission. Metronidazole only kills invasive trophozoites but has low cysticidal activity, which is consistent with the continued use of luminal agents after systemic therapy (11, 12, 21). This therapeutic gap underlines the need to explore compounds and delivery systems with wider spectrum activity across parasite life stages.

Current drugs and therapeutic limitations

Metronidazole is considered to be most commonly used drug for amoebiasis before invasive phase. It is a prodrug that relies on reductive activation via anaerobic or microaerophilic conditions. The activation of these metabolites causes damage to DNA and normal cellular processes, resulting in the death of trophozoites (13, 22). Tinidazole and equivalent nitroimidazoles possess similar mechanisms of action, may provide shorter courses in certain contexts, however they do not adequately circumvent the inherent limitations of nitroimidazole-based therapy (11, 12). The key limitations of current therapy are its adverse effects, poor activity against cysts, risk of drug interactions, intolerance to prolonged treatment and fears regarding resistance. Interaction with GI, metallic taste and neurological complication play a vital role in reduced compliance particularly over longer duration of the treatment (13, 22, 23). This is especially relevant for concerns in endemic settings where repeated exposures, suboptimal treatment and reinfection can occur. Consequently, better antiamoebic treatment should seek increased potency, decreased toxicity, cyst-stage activity or enhanced penetration of infected tissues.

Metronidazole resistance and redox adaptation

Decreased activation of metronidazole and altered redox metabolism primarily account for reduced susceptibility to this drug. Activation of metronidazole is dependent on low-redox-potential pathways including pyruvate: ferredoxin oxidoreductase, ferredoxin, nitroreductases and similar reductive enzymes (24, 25, 41). Less drug activation may lead to fewer toxic radical formation and less parasite killing. In vitro, *E. histolytica* with moderate metronidazole resistance or decreased susceptibility displays enhanced antioxidant defenses that include higher levels of iron superoxide dismutase and peroxiredoxin expression as well as lower levels of ferredoxin and flavin reductase (24). Further metabolic contributions to metronidazole activation, such as a malate enzyme pathway, have since been discovered through additional studies (22, 23, 42), and clinical studies have continued to identify slow rising inhibitory concentrations observed among amoebic liver abscess isolates (24, 25). These results suggest that decreased susceptibility is multi-factorial and tightly coupled to redox adaptation. Crucially, identification of these bacterial nitroimidazole resistance genes in stool does not validate true *E. histolytica* resistance because even the intestinal bacteria may carry them regardless of the parasite (43). Estimation of antiamoebic resistance in this fashion, however, thus necessitates parasite-specific susceptibility tests, molecular confirmation and standardization of assay conditions. Mutator strains are one of the new genetic tools

AIMCJ. (2026),3; (2): 22-33
doi.org/10.58564/AIMCJ3.2.2026.281

that will assist us in detecting mutations and pathways involved in future drug resistance (44).

Redox metabolism of the parasite and its thioredoxin reductase

Thioredoxin reductase is a principal redox enzyme of *E. histolytica* physiology. Trophozoites are exposed to reactive oxygen and nitrogen species released by epithelial cells, neutrophils, and macrophages during invasion. Since the parasite does not have a conventional glutathione/glutathione reductase system, it relies predominantly on thiol-based redox metabolism consisting of thioredoxin, thioredoxin reductase, peroxiredoxin, rubrerythrin, superoxide dismutase and NADPH-dependent oxidoreductases as well as low-molecular-weight thiols (14-17). The enzyme EhTrxR is responsible for the NADPH-dependent reduction of thioredoxin, a crucial event for peroxiredoxin-mediated detoxification and repair of oxidative damage (24,25). In addition, structural and biochemical data suggest that the parasite enzyme has unusual characteristics compared to mammalian thioredoxin reductase (17, 18, 45), which could enable selective inhibition. Since redox balance plays an important role in parasite survival, virulence, and drugs response such as metronidazole it is feasible that targeting EhTrxR would overcome some disadvantages of other nitroimidazole-based medicines. The role of redox regulation in *E. histolytica* biology is reinforced by recent studies on adaptation to redox-targeted pressure with Auranofin had demonstrated that parasite growth, virulence, and sensitivity to oxidative stress was affected by prolonged pressure (28). Experimental evidence has demonstrated that reactive sulfur species also impact on parasite viability, motility, and biofilm-related activity. These findings challenge the former view of redox metabolism as a minor metabolic pathway and support the conclusion that this form of metabolism is closely tied to parasite fitness and pathogenicity.

Auranofin and related redox-targeted inhibitors

Auranofin is a gold compound used originally for the treatment of rheumatoid arthritis and was identified as a promising antiamoebic compound via high-throughput screening (27). It is associated with the disruption of thioredoxin reductase-dependent redox homeostasis, however structural studies indicate that blockade of the catalytic site may be less straightforward than direct inhibition (26). *E. histolytica* virulence-associated pathways are modified by auranofin exposure that leads to oxidized proteins (28, 46). In contrast, the value of auranofin is in its proof-of-concept for redox-targeted therapy. Conversely, for utilization against amoebic infection we must evaluate selectivity, pharmacokinetics, toxicity, and drug combinations and resistance adaptation before clinical application. Additional redox-linked targets like adenosine 5-phosphosulfate kinase, peroxiredoxin, and cysteine metabolism pathways are also suggested for a more universal approach to therapy (29, 46, 47). New inhibitors will need to be evaluated with recombinant enzyme assays, trophozoites cultures, measures of host-cell toxicity, and appropriate in vivo systems.

Nanoparticle-based antiamoebic strategies

Nanoparticles represent a promising platform to promote the development of antiparasitic therapy by enhancing solubility, protecting unstable drugs, providing controlled release, facilitating tissue penetration, and reducing systemic toxicity (19, 20, 33, 34). Metallic nanoparticles might also exert direct antibacterial activity through the disruption of membranes, protein

AIMCJ. (2026),3; (2): 22-33
doi.org/10.58564/AIMCJ3.2.2026.281

interaction, and induction of oxidative stress (33, 34).

Direct evidence for *E. histolytica* is still rare but also promising. According to past studies, silver nanoparticles were responsible for decreasing trophozoite viability and inducing apoptosis-like cytomorphological changes (35); while experimental models showed that both, silver and copper oxide nanoparticles could reduce cyst viability (36). Therefore, the most rational future application for nanoparticles as related to *E. histolytica* may be in redox-targeted compounds as opposed to being nonspecifically toxic agents. The use of biodegradable polymeric nanoparticles, liposomes, and surface-modification nanocarriers could specifically deliver either more selective auranofin analogues or other compounds that inhibit EhTrxR to the intestinal or hepatic (liver) sites of infection. Nanoparticle activity is dose and route of administration dependent, as well as time-dependent with respect to their size, charge, coating type, and duration of in vivo exposure. Thus, standardized characterization should perform, using methods such as dynamic light scattering (DLS), electron microscopy (EM), zeta potential analysis, FTIR, and stability testing prior to the biological interpretation of the results (40). The mechanism or formulation strategy proposed for generalized formulations may be explained by evidence generated from other protozoa such as *Naegleria fowleri*, *Acanthamoeba castellanii* and *Cryptosporidium parvum* (35, 37-40).

However, these organisms are different in biology, life cycle, and host–pathogen interaction from *E. histolytica* to a considerable extent. Consequently, this review only mentions such studies as indirect supporting evidence as opposed to proof of anti-*E. histolytica* efficacy.

Discussion

The implication of this narrative review is that future antiamoebic therapy should not rely on the simple substitution of metronidazole with yet another broad-acting agent, but rather be developed upon species-specific weaknesses of Entamoeba spp. Metronidazole is still an important drug in the clinical setting of invasive amoebiasis, but its non-cysticidal activity, adverse effects, drug interactions, and reliance on reductive activation restricts it as a long-term therapeutic solution (11-13, 22, 23, 42). Thus, novel therapeutic approaches need to be developed targeting at the same time trophozoite survival and transmission stages.

Given the heavy reliance of *E. histolytica* on thiol-dependent antioxidant systems during tissue invasion and exposure to host oxidative stress, targets in redox metabolism represent some of the most promising therapeutic approaches. Besides virulence and drug response the thioredoxin reductase pathway has been linked with tolerance to oxidative-stress (14-18). Redox directed therapy may influence *E. histolytica,* providing proof of concept (27) for Auranofin but adaptation studies also demonstrate the potential of the parasite to induce more global stress-response pathways when under redox pressure (21, 27, 28).

Hence, future EhTrxR inhibitors should be evaluated for not only enzyme inhibition but also parasite selectivity, cytotoxicity potential, resistance development and in vivo efficacy using animal models of infection. Systems transporting drug molecules by using nanoparticles might be a strategy of auxiliary antiamoebic therapy through enhanced delivery, stability, solubility, controlled release and localizing the local concentration of drugs. Silver and copper oxide as

AIMCJ. (2026),3; (2): 22-33
doi.org/10.58564/AIMCJ3.2.2026.281

metallic nanoparticles may also have direct antiparasitic action, probably through oxidative stress and membrane damage; however, toxicity and reproducibility are major drawbacks with them (16, 17, 28-31). Carrier-based nanomedicine could provide a more secure translation if it optimizes delivery of therapeutically-relevant redox-targeted agents while minimizing host exposure (16, 17, 35). However, due to the diversity of nanoparticle synthesis methods, sizes, coatings, and concentrations as well as assay design among various studies it is difficult to compare them directly. Moreover, caution should be exercised when extrapolating data from other protozoa because they cannot substitute labor-intensive direct studies on *E. histolytica* clones.

The most feasible clinical strategy for the near future is probably rational combination therapy in which first generation antimicrobials such as metronidazole or tinidazole are paired with either redox-modulating agents or nanoparticle-based delivery systems. Because drug interactions may be synergistic, additive or antagonistic with respect to redox biology and with regard to the parasite stage being acted upon, such combinations must be evaluated carefully. Dosage-response, checkerboard assays, host-cell toxicity tests, and evaluations of drug activity in trophozoites and cysts along with in vivo models of amoebic intestinal and hepatic disease should be conducted in future studies. Characterization of nanoparticles in a standardized manner (particle size distribution, zeta potential, morphology, coating chemistry, drug-loading efficiency-release kinetics, and stability) is also required prior to clinical translation. Finally, linking therapeutic development to correct diagnosis. Accurate separation of pathogenic *E. histolytica* from nonpathogenic Entamoeba spp. is required to ensure appropriate treatment and as estimates of true therapeutic failure. Furthermore, new antiamoebic agents should also be affordable, orally bioavailable, room temperature-stable, and suitable for use in endemic areas. Those are practical requirements as critical to moving new therapies from the laboratory bench to public health application along with molecular potency. This study is a narrative review which does not conduct meta-analysis or estimate pooled effect sizes. The nanoparticle literature is heterogeneous, and good direct evidence for *E. histolytica* remains elusive. Studies with other protozoa are presented only to suggest potential mechanism and delivery possibilities but not infer similar efficacy in *E. histolytica*. Some of the experimental results are derived from in vitro models which need to be validated with standardized animal models and clinically relevant parasite isolates.

Conclusion

This narrative review concludes that *E. histolytica* is still an important causative organism of intestinal and extraintestinal amoebiasis. Even while metronidazole is still gold standard for treating invasive diseases, there are a number of reasons to support other treatment approaches, such as side effects, a lack of cysticidal efficacy, and concern about the development of decreased susceptibility.

Parasite redox biology, specifically the metabolism in conjunction with thioredoxin reductase, seems to represent a robust target as it is central to survival in and virulence against oxidative-stress, as well as drug resistance. Redox-directed therapy is also validated and strengthened via the synergistic action of combination treatment exemplified by auranofin and related inhibitors, while nanoparticle-based systems may enhance lead delivery, stability and selectivity.

AIMCJ. (2026),3; (2): 22-33
doi.org/10.58564/AIMCJ3.2.2026.281

Among the perspectives for the future, we believe that the best potential represents a combination of selective redox-targeted inhibitors with safe and well characterized nanocarriers. These approaches would benefit from standardized in vitro testing, host-cell safety assessment, pharmacokinetic studies, and in vivo validation prior to clinical translation.

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