Use of nanoparticles, a modern means of drug delivery, against cryptosporidiosis

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ABSTRACT
Cryptosporidium is a primary cause of waterborne epidemics, despite being previously considered only an opportunistic pathogen. The disease is associated with significant economic losses in humans and animals that are brought on by diarrhea, which frequently causes dehydration. Contact with diseased people or animals, as well as polluted water, is the major cause of infection. Different drugs are used to control the parasites. Nitazoxanide (NTZ), which is an anti-protozoan and anti-viral drug, is used to control helminths, viruses, and protozoan parasites as a broad-spectrum antibiotic and has been approved by the food and drug authority (FDA). However, the problem is the development of resistance over a period of time in these parasites. Nanoparticles have received significant attention as possible anti-parasitic agents in recent years. By directing medications to specific cellular locations, targeted drug delivery minimizes the side effects of medications. Nanoparticles have demonstrated effectiveness against different Cryptosporidium species. Nanoparticles loaded with NTZ are found to be an effective remedy for C. parvum in young ones and decrease the oocyst count shed in the stools. Additionally, silver nanoparticles have been proven to be effective against C. parvum by releasing silver ions that breach the cell wall of the oocyst, causing the escape of intracellular contents and the destruction of sporozoites within the oocyst. Implementing tiny particles for the purification of consuming water from Cryptosporidium is an economical and environmentally sustainable process. However, the use of nanoparticles in medicine requires more research.

INTRODUCTION
Cryptosporidium parasites infect the digestive systems of many vertebrates. They are grouped into the phylum Apicomplexa. Cryptosporidium (C) causes diarrhea, and the disease is referred to as cryptosporidiosis [1–3]. With Cryptosporidium, 41 species and more than 60 genotypes are documented [4–6]. According to reports, Cryptosporidium is the second most common reason why newborns under the age of two get mild or serious diarrhea, behind rotavirus [7]. Cryptosporidiosis has public health significance because Cryptosporidium species are notorious for diarrhea caused by traveling [8,9] and are also accountable for diarrhea epidemics associated with water resources, e.g., waterparks, and municipal water supply [10,11]. In consumers, C. hominis and C. parvum are among the most common organisms, although C. parvum also infects ruminants [12]. The organisms C. hominis and C. parvum result in approximately a million human fatalities annually [13]. C. parvum is claimed to cause numerous financial losses due to its high mortality rates, lower efficiency, and costly medical care [14]. In developing countries, infection negatively affects malnourished children, retards their growth, causes hindrance in weight gain, and impairs physical development. The impact of cryptosporidiosis on animals can differ based on factors such as species, age, and overall health. In typical animals, the
infection tends to be self-limiting and resolves within a week. However, in animals with weakened systems and pregnant animals, the infection may have more severe consequences, like weight loss, dehydration, and potentially even mortality [15].

This protozoon impacts not just individuals and wildlife but also a variety of birds. *C. meleagridis*, *C. baileyi*, and *C. galli* are pathogens that cause disease specifically in birds [16]. *C. meleagridis* and *C. galli* impact the digestive system and cause different levels of enteritis. *C. galli* infects and develops lesions in the proventriculus of poultry [17,18]. *C. baileyi* shows different clinical forms of the disease: respiratory, enteritis (digestive), and renal. It develops lesions in various organs like the gut, the kidneys, urinary tract, trachea, bronchi, air sacs, nasopharynx, conjunctiva, and bursa of Fabricius. Among birds, infection is frequently responsible for higher rates of death and illness [19,20] and has great economic importance. The life stages of *Cryptosporidium* are complicated and involve a single host for both sexual and asexual stages. Thick-walled oocysts are resistant to adverse environmental conditions and contaminate the water sources that are accidentally ingested by humans, animals, or birds [21,22]. The acidic environment of the intestine stimulates the excystation of oocysts, and sporozoites are released [23,24]. Sporozoites attach to the host’s cells of epithelial tissue and cause a parasitophorous vacuole to form. The parasite passes through different developmental forms like trophozoite, Type I meront, Type I merozoite, Type II meront, and Type II merozoite [1,25,26]. Type II merozoite forms microgamont (male) and macrogamont (female), which are round in shape and vary in size from 4 to 6 µm [27,28]. Microgametes fertilize the nearby macrogamont, which develops into a diploid zygote. Within the intestinal lumen, these zygotes develop into oocysts, which either re-infect the same host or may be excreted from the body through feces into the external environment.

Treatment of disease involves various drugs like nitazoxanide (NTZ), paromomycin, azithromycin, a combination of azithromycin and paromomycin, and rifaximin. NTZ is a US Food and Drug Administration (FDA)-approved drug whose active chemical is nitrothiazolyl-salicylamide [29]. The drug is an anti-protozoan/anti-viral agent that has wide-spectrum antibacterial activity against viruses, bacteria, helminths, and protozoan parasites [30–32]. It is effective in mild infections but does not give good results in moderate and heavy infections [33,34]. Paromomycin belongs to the group aminoglycosides, and it is found effective in children and adults to treat different levels of infection [35–37]. Paromomycin decreases the protozoa and oocysts shedding in stools and treats diarrhea. Though it does not eliminate the infections, symptoms appear again in half of the patients [38,39]. Azithromycin is an antibiotic that belongs to the macrolide group and has been found effective in some infections [40–42]. Rifaximin is an FDA-approved antibiotic that is used to treat travelers’ diarrhea [43–45]. Unfortunately, its action is restricted to people who are most at risk for serious illness. In recent years, researchers have initiated numerous initiatives to identify secure and effective parasite therapies. Here, we discuss many novel targeted mechanisms for treating cryptosporidiosis, along with current and emerging therapeutic approaches.

**Need for nanotechnology**

In spite of primary research efforts, to this point, since most parasitic infections do not elicit a strong immune response, there is currently no vaccine that is successful against any of the majority of common parasitic illnesses. Subsequently, the use of anti-parasitic agents is a very important strategic tool for fighting parasitic infections [46,47]. However, researchers instituted anti-parasitic drugs more than 50 years ago. Furthermore, while some drugs are efficient, the majority of anti-parasitic medications fall well short of the modern definition of a "drug" when it comes to acceptability and treatment; the duration of the remedy, precision, and the consent of the patient [48]. In contradiction, the cost of the latest drug development and drug discovery against parasitic infections is extremely low in comparison to the many other fields of study. In actuality, just 1% of the 1223 novel medications that were introduced to the marketplace between 1975 and 1996 were created specifically for the treatment of tropical invading parasitic infections like trypanosomiasis, malaria, and leishmaniasis. Until 2000, researchers allocated just 0.1% of global health research funding to the search for anti-parasitic drugs, reflecting a lack of concern for infections caused by parasites. So, coming up with new ways to give currently available anti-parasitic drugs that make them more effective, more accurate, easier to tolerate, and able to treat a wider range of parasitic diseases is a great idea that might help stop the disease pandemic [49,50].

Given the commonness of parasitic diseases and the bad side effects that come with current anti-parasitic drugs, it is important to look into new drugs that are highly effective, don’t cause side effects, and are not too expensive. Certain limitations afflict conventional preparations such as suspension or emulsion. There may be a need for a few innovative providers who can perfectly meet the requirements of a drug-transporting device due to factors like excessive dosage and low accessibility, first bypass impact, intolerance, instability, and variations in plasma drug degrees. Currently, nanoparticle delivery machines have been proposed as colloidal drug carriers. Nanoparticles may show size-related characteristics that vary extensively from the
Nanoparticles

With time, nanoparticles have drawn great attention. Nanoparticles are the basic component of nanotechnology, and their dimensions vary between 1 and 100 nanometers (nm). Scientists synthesized nanoparticles from organic materials, metals like carbon, and metal oxides [53]. Nanoparticles are available in different dimensions, shapes, and sizes in addition to their material [54]. They can be single-dimensional, like graphene, two-dimensional in nature, like carbon nanotubes (CNT), three-dimensional, like gold nanoparticles, or zero-dimensional, like nanodots. Their shapes range from spherical to cylindrical, tubular, conical, hollow core, spiral, and flat. They can also be crystalline, with homogeneous, regular surfaces, or amorphous, with uneven surfaces [55]. Nanotechnology has a vast range of applications in medicine, cosmetics, crockery, electronic appliances, and the aerospace industry.

Types of nanoparticles

There are different kinds of particles organic, inorganic, and metal-based.

Organic nanoparticles

The polymers of ferritin, liposomes, dendrimers, and micelles are known as organic nanoparticles. These particular nanoparticles are biodegradable, stable, non-toxic, and capable of transporting drugs. The hollow centers of liposomes and micelles, referred to as nanocapsules, are radiation, light, and heat sensitive [56,57]. These special properties make them a good medium for the delivery of drugs. These types of nanoparticles are commonly used in medical fields because they are efficient. In targeted drug delivery systems, nanoparticles are administered to specific organs of the body. The properties of nanoparticles depend upon the composition of the material, shape, size, flexibility of the material, and surface quality [58]. Nanoparticles are composed of various materials, i.e., lipids, and a vast range of synthetic polymers of various compounds. The most popular artificial polymers that are utilized to create nanoparticles are poly (lactic-co-glycolic) acid (PLGA), dextrans, and polyanhydrides, while natural polymers include elastin-like polypeptides. The use of these nanoparticles depends on the method of preparation, their toxicity, and their compatibility with loaded drugs [59]. Nanoparticles are modified by binding with ligands to their surface, e.g., peptides, antibodies, aptamers, and then applied to specific tissues like cancer cells [60,61]. The efficiency of nanoparticles also depends on their shape and structure. The efficiency of rod-shaped nanoparticles on targeted tissues is higher than that of spherical nanoparticles [62,63].

Inorganic nanoparticles

There are no carbon atoms in nanoparticles that are inorganic. They are further categorized into metal-based and metal oxide-based nanoparticles. Metal-based nanoparticles are prepared by converting the metals into nanoparticles either through constructive or destructive methods. All kinds of metals can be used for the formation of nanoparticles [64]. Nanoparticles are synthesized commonly from gold (Au), silver (Ag), zinc (Zn), lead (Pb), copper (Cu), cobalt (Co), cadmium (Cd), and aluminum (Al). These nanoparticles have specific characteristics like high surface charge, pore size, surface area-to-volume ratio, and surface charge density. Characteristics of different nanoparticles vary; e.g., aluminum nanoparticles show sensitivity to heat, sunlight, and moisture, have a large surface area, and are unstable [65]. Furthermore, silver nanoparticles exhibit lower reactivity and can serve as effective disinfectants and antimicrobial agents [66]. Gold nanoparticles are highly reactive and unstable [67]. Copper nanoparticles (CuNPs) are flammable solids, conductors of heat and electricity, and ductile [68]. Zinc nanoparticles are resistant to corrosion, can be employed in treating bacterial and fungal infections, and provide protection against ultraviolet radiation [69]. Cobalt nanoparticles are highly unstable, toxic, and can absorb magnetic waves and microwaves [70]. Metal oxide nanoparticles are formed by the modification of their corresponding metal-based nanoparticles. At room temperature, nanoparticles of iron (Fe) oxidize to iron oxide (Fe$_2$O$_3$) in the presence of oxygen. Metal oxide nanoparticles are formed when there is an increase in their reactivity and efficiency [71]. Aluminum oxide (Al$_2$O$_3$), cerium oxide (CeO$_2$), iron oxide (Fe$_3$O$_4$), magnetite (Fe$_3$O$_4$), silicon dioxide (SiO$_2$), titanium oxide (TiO$_2$), and zinc oxide (ZnO) [72–75]. In contrast to the corresponding metals, these small particles have unique characteristics.

Carbon-based nanoparticles

Carbon atoms are an integral part of carbon-based nanoparticles. Graphene, fullerenes, nanotubes of carbon...
(CNT), carbon black, carbon nanofibers, and carbon that are activated are the different types of carbon nanomaterials. Fullerenes ($C_{60}$) are composed of carbon atoms. Fullerenes molecules are round in form. There are about 28–1,500 atoms of carbon aggregated with each other by sp² hybridization to form fullerenes. The diameters of the single-layered and multi-layered fullerenes are 8.2 nm and 4–36 nm, respectively [76]. It is a carbon allotrope. The diameter of a graphene sheet is approximately 1 nm. It is a two-dimensional, hexagon-shaped honeycomb structure network composed of carbon atoms [77]. CNTs are formed by winding a graphene nanofoil with a honeycomb-like shape made of carbon atoms into elongated cylinders. Scientists form CNTs by winding the graphene nanofoil into elongated cylinders. A single-layer CNT has a diameter of 0.7 nm, while multi-layered CNTs have a diameter of 100 nm. CNTs range in dimension from various millimeters (mm) to a few micrometers (μm). Half-fullerene molecules may seal both ends of a CNT. This material—nanofoils wound into an oval or cup-shaped substitute for standard cylindrical tubes—makes up carbon nanofiber. Nanotubes of carbon are comparable to these. Made of carbon, carbon black is a kind of amorphous substance. The size of it ranges from 20 to 70 nm, giving it an elliptical form. Because of the particles’ strong reactions and interactions, carbon black particles were created [78].

**Applications**

Numerous uses have developed for nanoparticles but they are mostly desirable in medicine and drug delivery systems.

**Medicine**

Nanotechnology is a recent advancement in the medical field. Nanoparticles deliver drugs specifically to body cells [79]. We can minimize the side effects of drugs by placing them in a specific area. This method decreases the amount of drug, cost, and side effects. Nanotechnology helps in tissue engineering and reproduction. Nanotechnology can be used to replicate broken tissue and reconstruct it, a process known as the tissue engineering method. Tissue engineering has replaced the traditional methods of treatment, e.g., artificial implants and organ transplants. The development of bone-based CNT scaffolds is a particularly notable example [80]. Nanoparticles speed up the healing process, so they can be used as antiseptics, e.g., silver nanoparticles. Silver nanoparticles are also used to control bacteria and fungi and act as an antibacterial agent. Silver nanoparticles are highly effective against bacteria and fungi, which are resistant to drugs, and their sensitivity varies according to each species [81]. These nanoparticles show high efficacy against MRSA (methicillin-resistant *Staphylococcus aureus*), *Streptococcus pyogenes*, and methicillin-resistant *Staphylococcus epidermidis*. Silver nanoparticles break down the cell walls of these bacteria, e.g., *Klebsiella pneumonia* and *Salmonella typhi*. So, they are less effective against them [82]. A combination of chitosan (CS)-Ag nanoparticles has high potency to fight against *Escherichia coli* and is less effective against *Candida albicans*, which is due to variations in the structural composition of the outer wall of the cell and the presence of different functional groups around the cell wall of various types of bacteria [83]. Ag NPs work by attaching themselves to cells, breaking down enzymes and nucleic acids, and producing harmful free radicals and reactive oxygen species (ROS) that cause oxidative stress [84–87]. They function by inhibiting the synthesis of biofilms and the destruction of bacteria in previously formed biofilms [88]. The combination of Ag NPs and Quercetin has a synergistic effect and is also safe to use as an antibacterial against *E. coli* and *S. aureus* [89].

**Drug delivery system**

Nanoparticles are utilized for the delivery of different medicines to target sites. The antibacterial effect of drugs can be enhanced by their loading and conjugation with the nanoparticles. *Naegleria fowleri*, the causative organism of meningoencephalitis, can be cured by using Amphotericin B, Fluconazole, and Nystatin loaded on Ag NPs [90]. The increase in the efficacy of drugs is due to an increase in their total amount and their availability in the targeted tissue. The toxicity of Ag NPs can be reduced by the slow release of silver ions in the host cells [91]. Ag NPs loaded with oseltamivir are highly effective against influenza virus strain H1N1 because the antiviral activity of oseltamivir is enhanced due to the generation of ROS [92]. Silver nanoparticles can also be used to control Chagas disease, which results in extensive necrosis caused by *Trypanosoma cruzi* prepared from the reducing agent Xylan [93]. Drugs conjugated with silver nanoparticles show more efficiency than drugs used alone and are also less harmful and toxic to host tissues [94]. Silver nanoparticles are synthesized using plant hexane extracts from *Phaselous coccinus* [95]. Figure 1 illustrates the applications of nanotechnology in different fields, i.e., gene therapy, tissue engineering, drug delivery, biosensing, anti-parasitic antimicrobials, wound healing, cartilage repair, and bone repair.

Scientists have been using nanoparticles for various purposes in recent years, particularly as anti-parasitic agents [96–99]. In medicine, the extensive use of Chitosan Nanoparticles (CS NPs) is due to their compatibility with the environment and their bacteriostatic characteristics [100,101]. CS particles are prepared from N-acetyl-d-glucosamine and d-glucosamine subunits. Chemically, CS
NPs are polysaccharides that are formed by the deacetylation of chitin in a basic environment. They have no adverse effects, can be used as antitumor, antibacterial, and antifungal agents, are involved in wound healing, and act as immune system stimulants [102,103]. In nanomedicine, CS NPs are most preferably employed for the loading of drugs due to their specific characteristics for carrying drugs, which also increase the availability of drugs at target sites and the duration of their action. They can solubilize in an aqueous medium and produce positively charged ions. This property allows for their most common and efficient use with greater success [104]. It also increases the permeability of molecules through various surfaces, like mucosal surfaces [105]. The oocysts of Cryptosporidium are resistant to the environment, have high stability, and continue to thrive for a longer duration of time, up to 12 months, which is why they produce most waterborne diseases [106]. The oocyst wall of Cryptosporidium is very resistant and hard, and it requires a longer duration of time for the action of CS NPs for their complete elimination. There is a positive charge on the surface of CS NPs and a negative charge on the oocyst wall of Cryptosporidium, which is why they stick to each other firmly, and this phenomenon increases the duration of action of nanoparticles, which is highly toxic for Cryptosporidium oocysts. They interact with nucleic acids, disrupt their helical structure, and also produce ROS that impose oxidative stress and lead to oocyst inactivation [107,108]. Figure 2 explains the mode of action of CS NPs.

NTZ causes a remarkable decrease in the shedding of oocysts in immunosuppressed and immunocompetent mice. On the 11th day, the percentage decrease in oocyst passing in immunocompetent and immunosuppressed groups is 42.01% and 32.42%, respectively, and on the 19th day, the reduction percentage is 57.1% and 41.06%, respectively [30]. CS NPs loaded with NTZ are a very effective remedy for C. parvum in young ones and decrease the number of parasites shedding in the environment [109]. Conjugation of CS nanosuspension with Bupravaquone will increase the duration of the drug in the intestine and its availability, which is why this combination is more effective as compared to Bupravaquone alone [110,111]. The mucoadhesive property of CS NPs is responsible for enhancing the duration and improved action of drugs in the digestive tract and decreasing their excretion from the gut. In the gastrointestinal tract, CS NPs attach to the intestinal wall and attack the pathogen directly [112]. Praziquantel loaded on CS NPs is a very effective treatment for larval and mature stages of Schistosoma with a half-dose rate [113].

C. parvum continues to shed oocysts for 30 days in Swiss albino mice [114]. It is also reported that oocysts can be shed for up to 3 weeks in this study [115]. When severe
infection occurs with *Cryptosporidium*, watery diarrhea is tenacious and lasts for five weeks [116]. It is reported that CS NPs along with *Nigella sativa* (black cumin) reduce the shedding of oocysts and limit their spread both in immunocompetent and immunosuppressed mice on the 27th day after infection, with a reduction percentage of 79.16% and 73.33%, respectively [111,117]. CS NPs reduce the shedding of oocysts at 18 days after infection in immunocompetent and immunosuppressed mice by 17.3% and 11.7%, respectively [111]. *N. sativa* has various therapeutic effects like antioxidant, neuroprotective, immunopotentiation, antiasthmatic, antitumor, anti-inflammatory, and antimicrobial [118]. It is revealed that the seeds of *N. sativa* act as phytotherapeutic agents against plasmodium and also have antioxidant properties [119]. *N. sativa* extracts, in combination with honey, can treat cutaneous leishmaniasis (CL) better than honey alone [120]. A combination of *N. sativa* with CS NPs decreases the number of parasites by 77.5% [113]. CS NPs result in a reduction of parasite counts in the brain, spleen, and liver by 6.42%, 17.66%, and 23.94%, respectively, in mice infected with the *Toxoplasma* RH strain [121]. CS NPs combined with polyvinyl alcohol are used to break the link between *Cryptosporidium* sporozoites and the cells of the intestine *in vitro* and CS NPs show high efficiency against *Cryptosporidium* [122]. CS NPs conjugated with NTZ decrease the number of deaths in immunosuppressed mice compared to the untreated control group [121].

Therapeutic effects of various drugs are determined by evaluating the different histopathological improvements in the liver, intestine, and lungs in intestinal and extra-intestinal forms of *Cryptosporidium* infection. In cryptosporidiosis, there is shortening and then destruction of the villi of the small intestine, intensive inflammation, ulcers on the mucosal surface, and complete loss of the brush border, so absorption of nutrients does not occur. Dysplastic changes in the intestine were also observed [114,123,124]. Specific toxins produced by pathogens adversely affect the epithelial cells and lead to the atrophy of villi and complete loss of brush borders [125]. NTZ alone can be used for the treatment, but it shows a mild improvement in the pathological condition of the intestine [126]. However, the combination of CS NPs with NTZ causes significant improvement in the pathological changes by rejoining the atrophied intestinal villi and also improving the liver picture. Immunocompetent mice show better results as compared to immunosuppressed mice [124]. Table 1 illustrates the efficacy of different nanoparticles against *Cryptosporidium parvum* oocysts.

**Use of silver nanoparticles (Ag NPs)**

Silver nanoparticles show high efficacy in fighting against various pathogens and advancements in the field of science. Ag NPs are used in medicine and are capable of controlling bacteria, fungi, and protozoa [139–141].
### Table 1. Efficacy of different nanoparticles against Cryptosporidium parvum oocysts.

<table>
<thead>
<tr>
<th>Nanoparticle</th>
<th>Country</th>
<th>Test used</th>
<th>Sample extracted</th>
<th>Concentration/dose rate</th>
<th>Results/efficacy</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silver nanoparticles</td>
<td>United Kingdom</td>
<td>Counting of oocyst, sporozoites, and empty shells by PCM.</td>
<td>Fecal sample</td>
<td>500 µg/ml</td>
<td>Reduction in oocyst count 83.3 ± 3% to 33.3 ± 17.5%.</td>
<td>[127]</td>
</tr>
<tr>
<td>Chitosan nanoparticles loaded with nitazoxanide</td>
<td>Egypt</td>
<td>Oocyst count by using Modified Ziehl-Neelson stain</td>
<td>Fecal sample</td>
<td>200 mg/ml CS NPs loaded with NTZ</td>
<td>The reduction percentage in oocyst is 75.7%.</td>
<td>[128]</td>
</tr>
<tr>
<td>Chitosan nanoparticles</td>
<td>Egypt</td>
<td>Oocyst shedding by Modified Ziehl-Neelson stain</td>
<td>Fecal sample</td>
<td>500 µg/ml 1500 µg/ml 3000 µg/ml 5000 µg/ml 7000 µg/ml</td>
<td>Oocyst destruction rate is 68.88% 86% 91% 99.3% 99.87% at different dose rate mentioned in previous column after 72 h of exposure.</td>
<td>[122]</td>
</tr>
<tr>
<td>Gold nanoparticles</td>
<td>Thailand</td>
<td>Oocyst count by HPF.</td>
<td>Stool sample</td>
<td>1cc</td>
<td>Oocysts reduce from 8.8 ± 1.2 oocysts/HPF to 4.7 ± 1.2 oocysts/HPF.</td>
<td>[129]</td>
</tr>
<tr>
<td>Silver nanoparticles</td>
<td>Egypt</td>
<td>Oocyst count</td>
<td>Water sample</td>
<td>0.1 ppm</td>
<td>Reduction percentage in oocyst count is 87% at 30 min of exposure.</td>
<td>[130]</td>
</tr>
<tr>
<td>Chitosan nanoparticles</td>
<td>China</td>
<td>Oocyst count</td>
<td>Fecal sample</td>
<td>1 mg/Kg/day</td>
<td>Reduction percentage in oocyst count is observed 75.54% (4275.90 ± 703 oocyst)</td>
<td>[131]</td>
</tr>
<tr>
<td>Silver nanoparticles</td>
<td>Egypt</td>
<td>Oocyst count</td>
<td>Fecal sample</td>
<td>5 mg/Kg/day</td>
<td>Oocyst reduced from 13733 ± 3885 at 14 days post-infection to 247 ± 94 at 28-day post infection.</td>
<td>[132]</td>
</tr>
<tr>
<td>Gold nanoparticles</td>
<td>Canada</td>
<td>Oocyst count</td>
<td>Fecal sample</td>
<td>25 µL</td>
<td>Significant reduction in oocyst count. The mean no. of oocysts per 10 mg stool is $10^6$. Statically significant reduction in oocyst count. Modification of excystation behavior is 90%.</td>
<td>[133]</td>
</tr>
<tr>
<td>Silver nitrate nanoparticles</td>
<td>America</td>
<td>Oocyst count</td>
<td>Stool sample</td>
<td>100 mg/L</td>
<td></td>
<td>[134]</td>
</tr>
<tr>
<td>CP2-NP-906</td>
<td>USA</td>
<td>Cell culture to estimate the anti-parasitic effect on sporozoites and intracellular forms.</td>
<td>Cryptosporidium infected cells.</td>
<td>Size of CP2-NP-906 is 100 to 300 nM for delivery of drug.</td>
<td>Reduction in the level of parasites is by 200-fold in cell culture.</td>
<td>[135]</td>
</tr>
<tr>
<td>Silver nanoparticles</td>
<td>Egypt</td>
<td>Oocyst count by SEM.</td>
<td>Fecal sample</td>
<td>0.54–1 mg.</td>
<td>LC50 for 3 h of exposure is 0.54–1 mg.</td>
<td>[136]</td>
</tr>
<tr>
<td>Copper nanoparticles (CuO)</td>
<td>Egypt</td>
<td>Oocyst count by SEM.</td>
<td>Fecal sample</td>
<td>0.72 mg.</td>
<td>LC50 for 3 h of exposure is 0.72 mg.</td>
<td>[136]</td>
</tr>
</tbody>
</table>

(Continued)
used to control various protozoa like *Leishmania*, *Entamoeba*, and *Toxoplasma*, which results in the production of ROS and leads to oxidative stress [142]. Moreover, nanoparticles are small, which provides a greater surface area and a longer duration of contact for binding to the bacteria. It leads to the slow release of silver ions and then triggers ROS [143]. Ag NPs act on *C. parvum* by releasing silver ions, which enter the oocyst by breaking the cell wall and causing the expulsion of all intracellular contents and the destruction of sporozoites within the oocyst. The use of nanoparticles for the purification of drinking water is a cost-effective and environmentally friendly procedure [127,144,145]. Ag NPs kill bacteria by exerting their antibacterial effect through cytotoxic and cell-inhibitory action [146]. Ag NPs are also used to control various protozoa like *Leishmania*, *Giardia*, *Entamoeba*, *Toxoplasma*, *Plasmodium*, and insect larvae and helminths [112,147–149]. Ag NPs decrease the spread of *Leishmania* parasites by blocking their metabolic action and the destruction of promastigotes [150]. There are various mechanisms by which AgNPs cause the destruction of the oocysts of *Cryptosporidium* species. Glycoprotein and lipophosphoglycan, responsible for virulence, are destroyed by the formation of ROS and consequently due to oxidative stress. This leads to the oocysts becoming inactive and may not cause parasitic infection [151]. The size of nanoparticles is very small, so they can readily move throughout the cellular membrane, lead to adverse effects on parasites, and result in their killing [152]. Ag NPs can cause toxic effects by binding with the molecules of DNA and destroy the double-helical structure by disrupting the cross-linkage of DNA strands, as shown in Figure 3 [153]. Nanoparticles also interrupt normal biochemical reactions [154]. Silver nanoparticles can decrease the number of oocysts and their duration of survival in the environment, which reduces their propagation to new hosts.

Table 2 describes the activity of oocysts of *C. parvum* exposed to the silver nanoparticles and the control group, which are not treated with Ag NPs for different durations of exposure. Mortality and activity of oocysts exposed to various concentrations of Ag NPs for different intervals of time and in the control group (*p* < 0.05) show significant variation. If the Ag NPs are exposed to oocysts for 1 to 4 h at a dose rate of 0.05 ppm, they show better results than if exposed for 30 min. The percentage reduction in oocyst activity at different exposure times (30 min at 1 ppm and 2 h at 0.05 ppm) is 97.3% and 78.3%, respectively [130]. This shows significant reduction percentages, which are given in the table.

Different concentrations of Ag NPs can be used to limit the viability of oocysts, as the maximum reduction percentage can be observed at a dose rate of 1 ppm and the minimum reduction percentage is reported at 0.05 ppm. It is described that a wide range of doses are used to render the oocysts inactive, from 0.005 to 500 µg/ml, in a dose-dependent manner [127]. Exposure to Ag NPs reduces their feasibility in feces and their further propagation [155]. Nanoparticles also disrupt the structure of the oocyst’s cell wall, rendering them inactive. It is reported that low-dose

### Table 2: Activity of oocysts of *C. parvum*

<table>
<thead>
<tr>
<th>Nanoparticle Description</th>
<th>Country</th>
<th>Test used</th>
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<th>Concentration/dose rate</th>
<th>Results/efficacy</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Indinavir loaded modified nanoparticles (Ab-TMR-IND-Np). Anti-<em>Cryptosporidium</em> IgG polyclonal antibody conjugated with tetramethylrhodamine labelled nanoparticle (D,L-lactide-co-glycolide).</td>
<td>Italy</td>
<td>Histogram of human cell line is examined by IFA.</td>
<td>HCT-8 cells (Human ileocecaladenocarcinoma tumor cell line).</td>
<td>50 µM Ab-TMR-IND-Np to the HCT-8 cells at the time of oocyst addition. 50 µM Ab-TMR-IND-Np added to cryptosporidium infected cells.</td>
<td>Complete inhibition of oocyst excystation and no infection occur. The reduction percentage of intracellular parasites depends upon the time of exposure i.e. 25%–30% after 24 h, 51% after 48 h, 67% after 72 h, and 70% after 96 h.</td>
<td>[137]</td>
</tr>
<tr>
<td>Chitosan NAG nanoparticles (N-acetyl-d-glucosamine units) and chitosan Mix</td>
<td>USA</td>
<td>IFA</td>
<td>Human ileocecal adenocarcinoma cells (HCT-8 cell line) and human colonic adenocarcinoma cells (Caco-2 cell line).</td>
<td>500 µg/ml of chitosan NAG and Chitosan mix.</td>
<td>Chitosan NAG reduces level of parasites in HCT-8 61.2% and Caco-2 44.1%. Chitosan mix reduces intracellular forms in HCT-8 78.8% and Caco-2 67.9%. Paromomycin sulphate reduces the level of intracellular parasites in HCT-8 44.7% and Caco-2 32.9%.</td>
<td>[138]</td>
</tr>
</tbody>
</table>

Abbreviations: Scanning electron microscope (SEM), High Power Field (HPF), Immuno fluorescent Assay (IFA), Phase contrast microscopy (PCM), HCT-8 cells (Human ileocecaladenocarcinoma tumor cell line).
Ag NPs with smaller sizes show more stimulatory effects than large-size nanoparticles. Ag NPs are available most commonly in the range of 8.2 to 42.1 nm. The toxic effects and availability of nanoparticles for the purification of water are changed by the presence of fecal material, organic contaminants, and heavy metals [127,156]. Higher concentrations of chlorides combine with the Ag NPs and form insoluble aggregates, which decrease the exposure time of the pathogen to the nanoparticles and reduce its antiparasitic effects against *C. parvum* [157,158].

### Use of copper and gold nanoparticle

CuNPs have anti-microbial activity against various types of bacteria, e.g., *Salmonella enterica*, *S. aureus*, *Campylobacter jejuni*, *E. coli*, *Listeria monocytogenes*, *Aspergillus niger*, etc. [159–161]. The copper oxide nanoparticles can be employed for the control of parasites with an inhibitory concentration (IC₅₀) of 0.13 mg/l for *Entamoeba histolytica* and 0.72 mg/l for *C. parvum* [155]. It is also reported that exposure to copper oxide nanoparticles for 180 min at a concentration of 0.6 mg/ml can render 97% of *Giardia*...
Echinococcus granulosus parasites inactive [162]. The mode of action of CuNPs involves their interaction with sulphhydryl groups, which then leads to protein denaturation, which ultimately results in the death of pathogens [160]. CuNPs decrease the permeability of the cell membrane, production of ROS, disruption of DNA molecules, protein denaturation, and lipid peroxidation, and hence prove toxic for bacterial pathogens [163]. Drugs kill microbes by using the programmed cell death phenomenon, also known as apoptosis, and use different types of caspase enzymes for killing [164]. Exposure to CuNPs at different concentrations leads to the stimulation of cell death by activation of caspase-3 activity in the protoscoleces of Echinococcus granulosus. CuNPs exposure for 48 h can inactivate the protoscoleces by inducing regulation of caspase enzymes of 20.5%, 32.3%, and 36.1% at dose rates of 250, 500, and 750 mg/ml, respectively [165].

The effectiveness of gold nanoparticle solutions as anti-infective agents is extensively elaborated. The utility of the improvement of novel antibacterial drugs is stated [166]. Furthermore, the expertise of its hobby in opposition to pathogenic protozoa is restricted. Thus, the writers conduct an initial examination to evaluate the impact of a gold nanoparticle solution on Cryptosporidium oocyst. The authors use this version to evaluate the impact of gold nanoparticles on cells, as in the previously posted papers. Briefly, the writer used the 30 fecal samples with Cryptosporidium oocyst for trial. Every fecal sample was divided into two parts by naive manipulation and combined with 1 cc of gold nanoparticles [167,168]. The authors evaluate the reduction in the number of Cryptosporidium oocysts in each group. At the start, the average number of Cryptosporidium oocysts per high-power field (HPF) is 8.8 ± 1.2 oocysts/HPF. The reduction of the implied Cryptosporidium oocyst quantity after checking may be discovered. The common change after manipulation in the naive and gold nanoparticle solution combined groups is 1.2 ± 0.5 oocysts/HPF and 4.7 ± 1.2 oocysts/HPF, respectively. There is a huge distinction in the reduction of the number of oocysts between these two groups (P < 0.05; t-check). This preliminary report would possibly suggest that a gold nanoparticle solution has an impact on the inactivation of Cryptosporidium oocyst. This will verify the idea that attaching gold nanoparticles to indinavir may enhance its in vitro efficiency against C. parvum [137].

Future perspective

Cryptosporidium is a parasite made up of protozoan cells that can seriously harm both humans and animals’ gastrointestinal systems. It is highly resistant to traditional water treatment methods and can survive in aquatic environments for extended periods, posing a significant public health threat [169]. Nanoparticles have demonstrated their potential as a tool in the fight against Cryptosporidium because of their distinct chemical and physical characteristics. Nanoparticles are able to enter the parasite’s cell membrane, disrupting its integrity and inhibiting its growth and reproduction [170]. One potential future perspective for the use of nanoparticles against Cryptosporidium is the development of nanoparticle-based water filtration systems. These systems could be used to remove Cryptosporidium and other pathogens from drinking water, improving public health outcomes [171]. Another potential application of nanoparticles is in the process of evolving targeted drug delivery systems for the treatment of Cryptosporidium infections. By encapsulating drugs within nanoparticles, it may be possible to lessen potential side effects and increase medication effectiveness, as well as increase drug stability and shelf life [172]. Overall, the use of nanoparticles against Cryptosporidium holds great promise for improving water quality and treating Cryptosporidium infections. However, further study is required to completely comprehend the safety and efficacy of these approaches and to develop practical and cost-effective solutions for their implementation [173].

Conclusion

In the past few years, it has been observed that nanoparticles, particularly CS-based and silver nanoparticles, are capable of delivering drugs to the target sites with more efficiency and have fewer side effects and toxicity. In this review based upon previous literature, it is revealed that various medicines embedded in CS and Ag NPs are highly efficient anticryptosporidial agents employed to control Cryptosporidium species due to an increase in their availability and longer duration of action at target sites. Ag NPs and CS NPs are effective alternatives to drugs and safer to use. Nanotechnologies help us find effective disinfectants that are safe to use and also less costly for the purification of water by killing C. parvum and various other parasites and bacteria. However, nanoparticles are costly, and more research is required to find their practical uses in clinics and to find out the dose rate and safe concentrations of CS NPs and Ag NPs for a specific duration of time to fight against this parasite.

List of abbreviations

C. Cryptosporidium; FDA, Food and Drug Authority; PLGA, Poly Lactic-co-Glycolic Acid; Au, Gold; Ag, Silver; Zn, Zinc; Pb, Lead; Cu, Copper; Co, Cobalt; Cd, Cadmium; Al, Aluminum; Al₂O₃, Aluminum oxide; CeO₂, Cerium oxide; Fe₂O₃, Iron oxide; Fe₃O₄, Magnetite; SiO₂, Silicon dioxide; TiO₂, Titanium oxide; Zno, Zinc oxide; CNT, Carbon
Nanotubes; mm, Millimeters; µm, Micrometers; nm, Nanometers; MRSA, Methicillin-Resistant *Staphylococcus aureus*; ROS, Reactive Oxygen Species; *E. coli*, *Escherichia coli*; CS NPs, Chitosan Nanoparticles; NTZ, Nitazoxanide; Ag NPs, Silver Nanoparticles; CL, Cutaneous Leishmaniasis; ppm, Parts per million; CuNPs, Copper nanoparticles; IC₅₀, Inhibitory Concentration; Au Nps, Gold Nanoparticle; HPF, High Power Field; SEM, Scanning Electron Microscope; IFA, Immuno Fluorescent Assay; PCM, Phase Contrast Microscopy; HCT-8 cells, Human ileocecal adenocarcinoma tumor cell line; TS-DHFR, Thymidylate Synthase-Dihydrofolate Reductase; Ab-TMR-IND-Np, Indinavir loaded modified nanoparticles.

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**Conflict of interest**

The authors declare that they have no competing interests.

**Author contribution**

Conceptualization—FAA, SSI, LAM, FEMS, and RZA; Data analysis—FAA, SSI, LAM, FEMS, and RZA; Manuscript writing (original draft)—TA, WQ; Review and editing—TA, and WQ.

**References**


