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**REVIEW ARTICLE**

# Nanotechnology-Based Interventions Against *Cryptosporidium*: Current Insights and Future Directions

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**Abstract**

*Cryptosporidium* is a primary cause of waterborne epidemics, despite being previously considered only an opportunistic pathogen. The illness is linked to substantial financial losses in both humans and animals due to diarrhea, which often results in dehydration. The main sources of infection are contaminated water and contact with sick humans or animals. Various medications are employed to manage the parasites. The Food and Drug Administration (FDA) has authorized nitazoxanide (NTZ), an antiviral and anti-protozoan medication that may be used as a broad-spectrum antibiotic to manage viruses, helminths, and protozoan parasites. But the issue is that these parasites gain resistance over time. In recent years, nanoparticles have drawn a lot of interest as potential anti-parasitic medicines. Targeted drug delivery reduces pharmacological adverse effects by delivering drugs to certain cellular sites. It has been shown that nanoparticles are efficient against many kinds of *Cryptosporidium*. It has been discovered that NTZ-loaded nanoparticles are a useful treatment for *C. parvum* in children and reduce the number of oocysts excreted in the feces. Furthermore, silver nanoparticles have demonstrated efficacy against *C. parvum* by releasing silver ions that penetrate the oocyst's cell wall, allowing internal contents to escape and destroying sporozoites inside the oocyst. Using microscopic particles to remove *Cryptosporidium* from drinking water is a cost-effective and sustainable method. However, further study is needed before using nanoparticles in medicine.

**Keywords:** waterborne diseases, drugs, resistant, nanoparticles, water treatment

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## 1. Introduction

*Cryptosporidium* (C.) is a ubiquitous protozoal parasite, and it belongs to the phylum Apicomplexa (1). *Cryptosporidium* is a zoonotic intracellular parasite that invades the epithelium of the gastrointestinal system and causes diarrhea, abdominal pain, nausea, and vomiting (2, 3). This disease is also known as cryptosporidiosis, and over 40 species have been reported of which 20 cause the infection in humans (4, 5). *C. parvum* and *C. hominis* are the two common species from which *C. parvum* has zoonotic importance (6, 7). The animal hosts of *C. parvum* are goats and cattle and it mainly infects the newly borne claves and causes severe diarrhea, impacting their growth and survival (8). Cryptosporidiosis disease shows illness levels from asymptotic to acute morbidity, which leads to high mortalities (9). Hence, the presence of *C. parvum* in claves is a major cause of zoonotic spread and economic loss (10). Because of high mortalities in humans and animals, scientists are focusing on its control and prevention so that this disease may be avoided in humans and animals (11). Control of *Cryptosporidium* depends upon the prevention (disinfection of contamination sources i.e., water) and treatment using chemical antibiotics (12). The control of *C. parvum* is difficult in humans and animals because stability of the oocyst in the environment (resistant to drought, water treatment procedure, freezing), large scale of final hosts, and low infective dose (13).

Many anti-cryptosporidium drugs are used such as nitazoxanide, azithromycin, metronidazole, paromomycin, bovine anti-cryptosporidium immunoglobulin, and spiramycin but these drugs show no effective treatment against disease (14). Nitazoxanide is the only drug approved by the US Food and Drug Administration (FDA), and its effects in immune-compromised people are not satisfactory (15). Moreover, a large number of vaccine trials are being conducted against cryptosporidiosis, however, it is very difficult to control because of its complicated life cycle and high frequency of host (16). Because of these reasons, alternatives including plant derivates, therapeutic, and nanotechnology are being suggested (17). All these drugs and alternative to control *Cryptosporidium* chiefly depend upon the proper delivery (18). Most drugs fail due to improper delivery, lack of accuracy, and wastage because of body metabolism (19). To overcome these issues, nanotechnology is being suggested. Nanotechnology has a wide range of importance in the medical field by acting as a treatment, preventive, and diagnostic tool (20). Nanotechnology consists of the formation of different types of nanoparticles (NPs) that have nanometre-sized structures and can affect the biological process (21). NPs are categorized into metallic oxide, non-metallic oxide, ceramic, etc. and they are synthesized in different sizes, shapes, and structures according to their use. NPs for their unique structural properties and features, can treat many infectious diseases (22). Medical and pharmaceutical industries have been revolutionized because of using NP-based products in the market (23). NPs perform several activities of medicinal importance including boosting the immune system as well as controlling several diseases by acting as a delivery agent (24). Many researchers have discussed the anti-protozoal activity of NPs on different parasites, *Toxoplasma*, *Giardia*, *Leishmania*, *Plasmodium*, *Entamoeba*, and *Cryptosporidium* (25–27). Because of their distinct chemical and physical properties, NPs have shown potential as agents against *Cryptosporidium*. They penetrate the membrane of parasite cells and prevent them from growing and reproducing (28). NPs inactivate the mechanism of *C. parvum* and decrease the number of oocysts and their survival in the environment (29). For the treatment of *Cryptosporidium*, NPs are used as a drug-delivery agent as they are encapsulated with drugs that increase their stability, durability, and efficacy (30). Additionally, cryptosporidiosis can be prevented by disinfection of oocysts of *Cryptosporidium* including *c. parvum* (31). An interesting feature of NPs is also that they can help in the diagnosis of *Cryptosporidium* because of their use in various molecular techniques (32). This review article highlights the role of NPs as diagnostic, disinfectant, and targeted drug-delivery agents against cryptosporidiosis. Nanoparticles induce anti-parasitic effects against *Cryptosporidium* through multiple pathways, including binding to nucleic acids, inhibiting enzyme activity, and compromising cell membrane integrity, which ultimately results in oocyst degradation and cell death as shown in Fig 1.

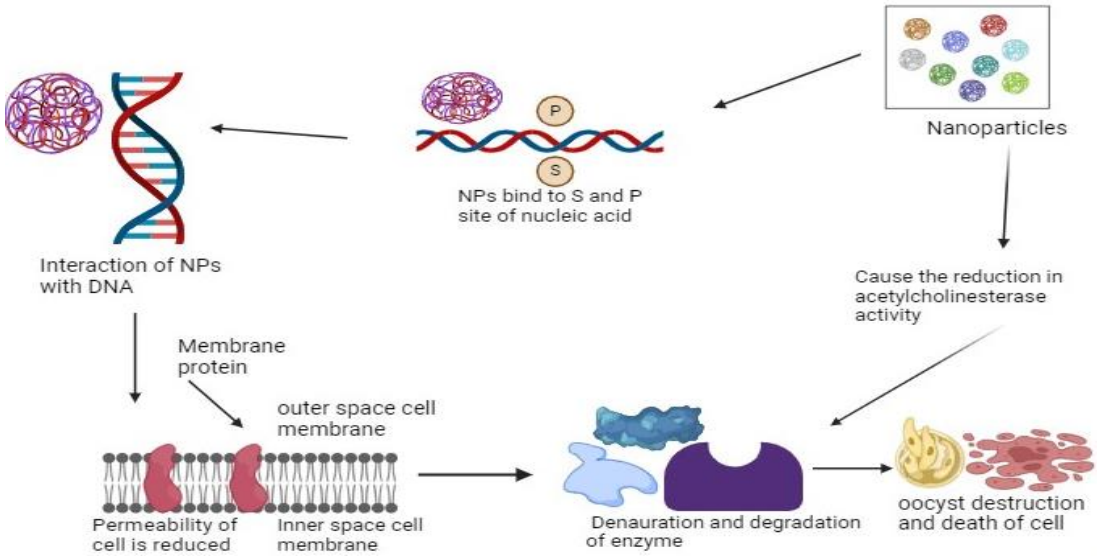


**Fig.1: Schematic representation of the mechanisms by which nanoparticles exert toxicity against *Cryptosporidium*, including DNA interaction, reduction of acetylcholinesterase activity, and cellular membrane damage leading to oocyst destruction.**

**2. Life cycle and Pathogenesis:**

*Cryptosporidium* has a direct life cycle, and it completes its sexual and asexual stages in a single host (33). The infected host (animals and humans) excreted the oocyst in the environment through feces and other routes such as respiratory secretion (34). When oocysts are ingested, they move to the small intestine and stomach then different factors like temperature, a pancreatic enzyme, bile salts, and carbon dioxide, trigger the excystation of the oocyst and release the four infective sporozoites (34). These sporozoites move smoothly over intestinal cells' surface and attach to intestinal epithelial cells' microvillus (35). The sporozoites move into the epithelial cell and the movement of these sporozoites depends on the apicomplexan parasite (36). Sporozoites of *C. parvum* undergo the movement of helical and circular, which depend on actin-myosin-tubulin (37). During the mobility, sporozoites secrete the protein (thrombospondin-related adhesive protein and mucin-like glycoprotein) and these are involved in invasion and attachment to the host cell (37). After the invasion, each sporozoite transforms into a trophozoite and forms a parasitophorous vacuole inside the host cell (38). Parasites are present inside the vacuole which is covered with a membrane originating from the host cell (39). The trophozoites multiply inside the parasitophorous vacuole and undergo asexual reproduction which initiates the merogony and produces type 1 meront having eight merozoites (40). These merozoites are released and adhere to the surface of the epithelium, where they go through further merogony to produce type 1 and type 2 meronts. In type 2 meronts, four merozoites are present (33). During the sexual cycle, the merozoites are released, again attached to the epithelial cell, and gametogenesis starts (41). Each merozoite produces macrogamonts (female) and microgamonts (male). Microgamonts differentiate and produce up to 16 microgametes, after undergoing nuclear division (42). These microgametes then join with macrogamonts to form a zygote. The zygotes formed as a result of this sexual reproduction differentiate either into thick-walled or thin-walled oocysts (43). Thick-walled oocysts breach the intestinal lumen and come into the environment through the feces, causing the infection into other susceptible hosts (44). Thin-walled oocysts cause autoinfection and excystation occurs when detached from epithelium, repeating the cycle (45). In this way, the life cycle continues. At the Sporozoite stage, the release of lactase hydrogenase, enhances the cell death rate, the disintegration of tight cell junctions, and a reduction of barrier function, these are all related to cell damage (46). This damage is also because of the direct disruption of host epithelial cells and may be indirect via the effect of cytokine and inflammatory cells on the site of infection (47). During *Cryptosporidium* infection, the mechanism that causes the cellular damage remains unknown; however, some molecules such as proteases, hemolysins, and phospholipases can destroy the tissue (48). The pathogenesis of cryptosporidiosis is triggered by the growth of *Cryptosporidium* within the epithelial cells, resulting in cell death (49). This caused damage to the intestinal barrier that led to vomiting, diarrhea, bloating, and intestinal pain (50). When infection occurs, the host stomach develops inflammation, and the gut lining becomes more inflamed and damaged when immune cells move to the infection site (51). In severe cases, the disturbance of the intestinal lining and deterioration of epithelial cells trigger malnutrition and inadequate absorption (52). Chronic infection can lead to persistent gastrointestinal symptoms that may cause dehydration, weight loss, and other losses (53). The symptoms of

cryptosporidiosis, such as diarrhoea and abdominal pain are caused by the immune system's reaction to the death of the host intestinal cells (54). Depending on the host's immunological condition, the severity of cryptosporidiosis may vary, immunocompromised people are more susceptible to suffering from a serious and prolonged disease (55).

## 3. Use of Nanoparticles against Cryptosporidiosis

Nanoparticles have been searched to be effective against cryptosporidiosis in three different ways. First, as a disinfectant of water to kill environmental oocysts, second as part of diagnostic tools for the detection of cryptosporidiosis, and third as a therapeutic delivery agent for treatment of *Cryptosporidium* infection. All of these uses are discussed in the following sections.

### 3.1. As a disinfectant of water

Many conventional methods are used for the disinfection of water; ozonation, chlorination, and ultraviolent treatment (UV) (56). Due to the low cost of chlorination, it is a globally used method for water disinfectant (57). When chlorine is used in high doses, it interacts with water-borne pathogens and forms a carcinogenic disinfection byproduct (DBP) (58). The ozonation is more costly than the chlorination method used for water disinfection, but it generates toxic bromate by reacting with water bromide ions. But all these methods have disadvantages, that make them unsuitable for disinfectant water (59). So due to all these demerits of methods, researchers move toward modern technique nanotechnology for water disinfection (60). Zinc oxide NPs (ZnO) are used to prevent water disinfection and overcome the production of BDP (61). Silver NPs also show a mechanism of protozoal inactivation by the action of silver ions. Silver ions enter the oocysts, destroy the sporozoites, react to the cell wall, and cause leakage (31). AuNPs and CuONPs show a toxic effect on *Cryptosporidium* cysts and oocyst walls (62). The time and concentration of nanoparticles greatly impact the mortality rate (63). Moreover, many researchers show an effective association between nanoparticles and oocyst walls, which may give rise to new tools for this material treatment and detection (64).

### 3.2. As a diagnostic tool

Different nanoparticles are used to detect *Cryptosporidium*. By using nanoparticles, we improve the specificity and sensitivity and overcome the demerits related to fluorescent labelling (65). Researchers developed the method of a colorimetric detection system for the detection of *C. parvum* oocyst (66). This system is used to detect mRNA molecules that are taken out from *C. parvum* oocyst (67). Gold nanoparticles (AuNPs) are also used to detect *C. parvum* oocyst in water samples by developing an electrochemical immunosensor technique based on a sandwich enzyme-linked assay (68). When applying this technique rather than standard ELISA, the detection sensitivity increased by 500 times (69). AuNPs can be filled in biosensor systems for detecting the *Cryptosporidium*. When antigens and antibodies of cryptosporidium are detected, this system provides the signal (70). Copper oxide (CuONPs) can be used in different techniques for the detection of *Cryptosporidium* such as optical and electrochemical detection, biosensor application, and colorimetric assay (71). Their unique properties make them essential tools for the detection of cryptosporidium, which include having the capacity to enhance the signal and their large surface area (72). However, limited studies are present on the application of AuNPs to detect *Cryptosporidium*, so further research is required to identify the use of AuNPs in this area.

### 3.3. For the treatment

Nanoparticles are used to treat *Cryptosporidium* by acting as nanomedicine and drug-delivery agents (73). *Cryptosporidium* nanoparticles are widely used as nanomedicine due to their distinctive properties of drug delivery that increase the availability of drugs at the target site and enhance the duration of their action (74). Because of their environmental sustainability properties, chitosan nanoparticles (CS NPs) are also widely used as antiparasitic agents (75). They increase the efficacy of drugs and can dissolve in the water, releasing positively charged ions (76). It also increases the penetration of molecules across a variety of surfaces including mucosal surfaces (74). *Cryptosporidium* oocyst wall is resistant to the environment, so due to this CS NPs take more time to completely eradicate them (77). They adhere to each other so tightly because of cryptosporidium oocyst wall has negative charges and positive charges are present on the surface of CS NPs, this increases the action of NPs which extremely damages the *Cryptosporidium* oocysts (78). Silver nanoparticles (AgNPs) show their toxicity through the release of silver ions which enter the oocyst through the cell wall and eliminate the sporozoites (79). Different mechanisms of Ag NPs cause the eradication of *Cryptosporidium* oocyst by destroying the double helical structure. This also causes the inactivation of oocysts by destroying the formation of ROS and reducing their survival time in the environment (80). Copper nanoparticles (CuNPs) cause the death of pathogens by interacting with sulfhydryl groups which leads to the denaturation of protein (81). Antiparasitic drugs can be delivered with ZnO NPs for the control of *Cryptosporidium* (82). ZnO NPs showed their function with drug molecules by acting at the target site to achieve desirable treatment which can increase the drug

efficacy and decrease the side effects (83). They are used in vaccine formulation and utilized as carriers to enhance the host immune response against *Cryptosporidium* (84). Gold nanoparticles (Au NPs) are also used as drug delivery agents by combining with nitazoxanide drugs and increasing their efficacy (85). AuNPs are also used in photodynamic therapy which has the potential to produce the reacting oxygen species (ROS) to combat the pathogen (86). Overall, using nanoparticles against *Cryptosporidium* significantly enhanced the water quality and treated the *Cryptosporidium* infection (87). However, more study is required to understand the efficacy and reliability of these nanoparticles and establish feasible, affordable alternatives for their application (88).

## 4. Limitation

Nanoparticles have great potential against different pathogens, including a protozoal parasite *Cryptosporidium*, which causes cryptosporidiosis. However, many limitations and difficulties are associated with implementing nanoparticles against *Cryptosporidium*.

## 5. Toxicity of Nanoparticles

Some NPs have a harmful impact on human cells and the environment. Due to their tiny size, they penetrate tissues, organs, and cells, which may cause accidental toxic exposure. NPs collect organs and tissue, having a detrimental effect. When nanoparticles are used for the treatment of water, then they are accidentally ingested and have a long-term impact on human health such as cellular damage, inflammatory response, and the development of cancer. It is also difficult to determine the appropriate balance between concentrations of NPs that are beneficial against *Cryptosporidium* and that are safe for the environment and humans. The toxicity of NPs against non-targeted organisms occurs when high concentrations of NPs are used against *Cryptosporidium*. Various NPs may come into contact with *Cryptosporidium* in different methods which might provide unequal results related to toxicity and efficacy. However, there are limited studies present on the mechanism of nanoparticle toxicity against *Cryptosporidium* and other organisms.

## 6. Cost of NPs

The cost of applying nanoparticles in several factors such as research and development, production, and implementation, to combat *Cryptosporidium* is expensive. A lot of research is required for the development of NPs that specifically target the *Cryptosporidium*. These NPs are designed with different properties such as material composition, surface charge, and size. The materials used for NPs synthesis including polymers, carbon-based materials, or metal (silver, copper, etc) are costly. Specific equipment, methods, and techniques are used for the manufacturing of NPs. These procedures need reliability and regulation, so they are expensive. The use of NPs for the treatment of water or other applications requires further manufacturing procedures. The expenses involved in implementing NPs into water treatment include installation, running, and maintenance of the water system. However, the management of NPs cost against *Cryptosporidium* with their advantages and efficacy has a significant role in practical and widely used.

## 7. Conclusion

Nanotechnology offers a promising and innovative frontier for the diagnosis, disinfection, and targeted treatment of *Cryptosporidium* infections, addressing critical limitations in current medical and environmental control methods. Despite the significant potential of nanoparticles to improve therapeutic efficacy and environmental safety, future research must rigorously evaluate their long-term toxicity and cost-effectiveness to ensure practical application. Ultimately, optimizing nanoparticle design and delivery strategies remains essential to overcoming existing challenges and mitigating the global impact of cryptosporidiosis in both human and animal populations.

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