Efficacy of Gold Nanoparticle-loaded with Nitazoxanide on Parasitological and Histopathological Parameters in Murine Cryptosporidiosis

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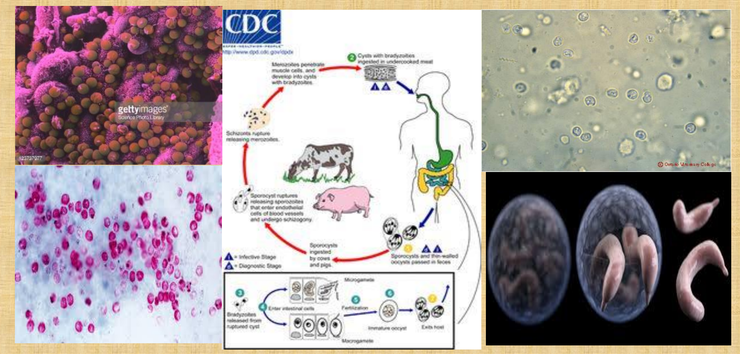
Abstract

Cryptosporidium parvum is a protozoan parasite that infects the gastrointestinal epithelial cells causing several parasitological and pathological changes. This work aimed to evaluate the effectiveness of Nitazoxanide alone or loaded with gold nanoparticles in treatment of cryptosporidiosis. **Methodology:** This study included five groups of mice: group I, infected control; group II, infected and treated with Nitazoxanide; group III, infected and treated with Gold Nanoparticle; group IV, infected treated with Nitazoxanide loaded with gold nanoparticles and groups V non-infected control. Mice were subjected to stool examination for oocyst counts prior to and after 2 weeks post infection and were later sacrificed for intestinal dissection and routine histo-pathological examination, beside measurement of fecal IgA in stool samples and different cytokines in serum samples. **Results**: Infected control group showed the highest numbers of oocysts shed compared to the other groups. The highest reduction of oocysts shed was observed in group of mice treated with Nitazoxanide loaded with gold nanoparticles 93.7 %. Low-grade dysplastic changes were seen in group of mice with combined treatment. The highest significant reduction of fecal IgA was observed in combined therapy. Mice with combined treatment showed a high significant ($P<0.001$) increase in serum levels of both IFN-γ and IL-10 and moderate significant ($P<0.01$) reduction in serum level of IL-5 when compared to group infected non-treated mice, while no significant difference between all treated groups and group infected non-treated mice in serum level of IL-2. **Conclusion:** This study was concluded that the combination of Nitazoxanide loaded with gold nanoparticles was effective in the treatment of *Cryptosporidium* infection.

Background

Cryptosporidiosis is a parasitic disease caused by Cryptosporidium. It is an intestinal parasite and is typically an acute short-term infection. It is spread through the fecal-oral route, often through contaminated water; the main symptom is self-limiting diarrhea in people with intact immune systems. The parasite is transmitted by environmentally hardy microbial cysts (oocysts) that, once ingested, exists in the small intestine and results in an infection of intestinal epithelial tissue. Multi-drug resistance is a growing problem in the treatment of infectious diseases. Thus, there is an urgent need to search for new alternatives for the treatment and control of infectious diseases, Gold and silver nanoparticles are now being considered as a potential source of novel antimicrobial agents, which offer several advantages such as broad spectrum activity and lower tendency to induce resistance (Rai *et al.,* 2009).



Objectives

This work aims to evaluate the efficacy of Nitazoxanide alone or loaded with Nano-gold nanoparticles for treatment of Cryptosporidium infection in murine cryptosporidiosis.

Methods

- **Parasites** Stool samples were collected from 30 immunosuppressed patients with chronic diarrhea. All samples were microscopically screened by direct smear, iodine smear and modified Ziehl–Neelsen acid fast stain (MZN), aiming to identify the positive cases of *Cryptosporidium.*
- **Synthesis of AuNPs** To a 500 mL round bottom flask, 250 mL of HAuCl4 were added, a condenser was adapted to the flask, and the solution was refluxed with stirring for 1 hr. using a sand bath. Then 25 mL of 38.8 sodium citrate solution (Sigma, Germany) were added. The flask was again left to reflux for 15 minutes. Then the solution was allowed to cool and stored in dark until further use.
- **Drugs** Nitazoxanide (Nanazoxid) (100mg) will be used.
- **Loading of Nitazoxanide to AuNP** Two nM of AuNP- solution was prepared from stock solution.The pH was adjusted to 7.4.100mg of Nitazoxanide was added to 20 mL of AuNP- (2 nM; pH = 7.4). Nitazoxanide -loaded nanoparticles were separated from aqueous suspension by centrifugation at 20,000 g and 14°C for 30 minutes. The supernatant was collected and protein content in supernatant was determined by the Bradford protein assay spectrophotometric method at 595 nm.
  - **Animal infection** The mice will be divided into different immunocompetent groups. Each mouse will be infected by oral inoculation with the isolated *Cryptosporidium* oocysts in a dose of about 10000 oocysts/ mouse **(Gaafar, 2007).**
- **Group I:** Infected control mice. **Group II:** Infected animals treated with Nitazoxanide orally (100mg/kg/ mice) daily for five consequence days two weeks post infection. **Group III:** Infected mice treated with Nano gold + Nitazoxanide orally (10 ng/kg/mice) daily for five consequence days two weeks post infection. **Group VI:** infected mice treated with Nano gold orally (100mg/kg/ mice). daily for five consequence days two weeks post infection. **Group V:** Normal mice. Animals were sacrificed four weeks post infection.
- **Parasitological examination:** Mouse fecal samples were collected prior to inoculation and after infection.
- **Acid-fast staining of fecal oocysts:** A commercially available acid-fast staining kit (Medical Industries Inc., Las Vegas, Nev.) was applied as recommended to fecal smears.
- **Histopathological examination:** The small intestine of mice were fixed in 10% neutral buffered formalin . Sections stained by hematoxylin and eosin (H&E) and (ZN stain) then examined by light microscopy according to standard operation procedures (Tziporie*t al*., 1981).
- **Cytokine production measurements**:IL-2, IFN-γ, IL-5, and IL-10 were measured in serum samples of different studied groups by commercial ELISA kits (R&D Systems, Minneapolis.

Results

Direct Smear

In bright-field microscopy using differential interference contrast (DIC), oocysts appear as small round structures (4 to 6 µm) similar to yeasts. They do not auto fluoresce.

Fig. (1) : Microscopic Examination of Cryptosporidium Oocysts .

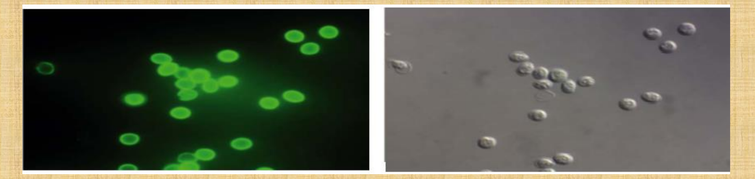


Table (1): Effect of treatment with Nano-gold loaded with Nitazoxanide on Cryptosporidium oocysts/ gm stool.

Mouse groups	Oocyst shedding (No./g stool) ± SD	% Reduction
Normal Mice	-	-
Infected Mice	24.9± 4.4	-
Infected treated with Nitazoxanide	8.2 ± 0.45	66.1 %
Infected treated with Nitazoxanide+Nano	4.6± 0.8	81.5 %
Infected treated with Nano	17.3 ± 1.23	30.5 %

Table (2): Detection of endogenous developmental stages of cryptosporidiosis

Mouse groups	Oocyst shedding (No./g stool) ± SD	% Reduction
Normal Mice	-	-
Infected Mice	88.9 ± 11.5	-
Infected treated with Nitazoxanide	32.1 ± 5.3	63.9 %
Infected treated with Nitazoxanide+Nano	19. 6 ± 1.9	78 %
Infected treated with Nano	53.1± 9.28	40.3 %

Histopathological examination

Figure (2): Histological sections of intestinal mice infected with *C. parvum*

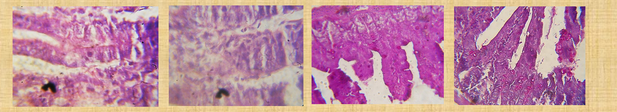


Table (3):- Measurement of IL-2, IL-5, and IL-10 in serum samples of different studied groups

Mouse groups	IL-2	IL-5	IL-10
Normal Mice	26.39± 3.28	22.12 ± 1.8	20.00 ± 2.71
Infected Mice	35.83± 4.52	252.02 ± 11.3	160.00 ± 12.34
Infected treated with Nitazoxanide	24.15 ± 1.2	123.53 ± 1.5**	238.11 ± 2.1*
Infected treated with Nitazoxanide + Nano	29.45 ± 2.30	217.25 ± 4.7	271.2 ± 3.3**
Infected treated with Nano	28.59 ± 1.90	162.91 ± 4.2*	398.72 ± 27.4***

Conclusions

Oocyst per gram (OPG) count showed an increasing trend in control (untreated) animals. Multiple dose of 10mg/kg body weight of Nitazoxanide caused a significant decrease in OPG count from 6thday post treatment and onward ($P<0.05$). High significant decrease ($P<0.001$) in relation to the efficacy of Nitazoxanide loaded with Nano-gold at 10mg/kg body weight in mice against Cryptosporidiosis.

Histologically, morphological and cellular alteration of microvillus membrane integrity revealed that administration of Nitazoxanide loaded with nano-gold ameliorated the mucosal damage in mice, compared with the severe microvillus damage, edematous and vacuolated epithelial cells in non-treated mice.

In Conclusion: This study was concluded that the combination of Nitazoxanide loaded with gold nanoparticles was effective in the treatment of Cryptosporidium infection under experimental conditions showed better results than Nitazoxanide alone and ameliorate the mucosal damage in mice.

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