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EFFECT OF COPPER NANOPARTICLES ON *CRYPTOSPORIDIUM* OOCYSTS IN VIVO

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Abstract

The aim of this research is to assessment the effect of copper nanoparticles inside the body by exposure the samples diagnosed using the modified acid fast stain and microscopic and isolated examination using the floatation into three concentrations per nano.material is(01, 0.03 and 0.05). And then examined the samples at half an hour after exposure using trypan blue stain half an hour later and was the evaluation of oocysts exposed to the same concentrations of nanomaterial's internally using laboratory mice, where mice were divided into five groups one groups, one of which had positive control and The other was negative control and the results in groups exposed to copper nanoparticles concentration (0.01,0.03 and 0.05)

Keyword :- *cryptosporidium* spp. , nanoparticles , copper , in-vivo

Introduction

Cryptosporidium spp. are apicomplexan parasites that inhabit the brush-borders of the gastrointestinal epithelium and infect humans and more to infect other vertebrates (Xiao & Cama,2018). Its includes protozoan parasites within the Phylum of Apicomplexa, although these parasites have lost the apicoplast which a is unique feature of apicomplexan organisms, they do not possess plastids or mitochondrial genome and there is only evidence of an atypical mitochondrion with no electron transport or oxidative phosphorylation function (Douvropoulou.,2017) The parasite able to infect the channel of humans and most vertebrate animals,it absolutely was the main responsible pathogen for 905 waterborne and 25 foodborne outbreaks worldwide (Efstratiou et al., 2017; Ahmed & Karanis 2018). Cryptosporidium is described to be second after rotavirus, reason for moderate to severe diarrhea during the primary 2 years of life (Roth et al .,2018). The species C. hominis and C. parvum are responsible for nearly 1,000,000 deaths in humans every year (Villanueva, 2017).

The main motive is the expectation that nanoparticles will be able to be used in the treatment of various diseases in the future (Angeli *et al*, 2008; Debbage, 2009). It was determined that through their unique properties and large surface areas, metal oxide nanoparticles possess effective antimicrobial activities (Elechiguerra et al, 2005). Particularly, owing to their great chemical reactivity, nanoparticles are capable of producing reactive oxygen species (ROS), which have the ability to kill infectious agents, The use of metal oxide nanoparticles exhibiting the antimicrobial activity offers the possibility of an efficient removal of pathogens from wastewater (Elechiguerra et al, 2005). The NPs may not have the pronounced antimicrobial activity when compared to the bulk formulations of the metal oxide or solutions of metal salts. But, the stability

and slow release of metal ions from nanoparticles are main characteristics which 594 give them the advantage in use (Heinlaan *et al*, 2008). The antimicrobial efficiency of NPs depends on the particle size (Adams *et al*, 2006). The smallest sized of NPs showed the strongest effect (Lu et al, 2013). Some researches indicate that silver nanoparticles, gold, chitosan, and oxidized metals have growth inhibitory or cytotoxic effect on various parasites, including *Giardia, Leishmania, Malaria, Toxoplasma* and insect larva (Saad *et al*, 2015).

Materials and methods

Samples collection

The samples collected from patients with diarrhea proved positive in AL-Batool hospital in Wasit government after direct examination, each stool sample was preserved in 2.5% potassium dichromate at 4 ºC until used.(Maryanti,2017).

Detection of *cryptosporidium* parasite

By using the Modified acid fast stain to detect the oocyst of cryptosporidium in the following steps :- taken one gram of stool sample placed on slide and fixed with heat and the smear is placed on a staining rack and flooded with carbolfuchsin for 3 min ,then the slide is placed on steam and allowed to stain for 5min. If the slide begins to dry, more stains added,The smear is rinsed with tap or distilled water and decolorized with3% acid alcohol solution for30s (thicker smears may require alonger time),and rinsed again with tap or distilled water and drained, and the slide is flooded with methylene blue counterstain for 1min, Rinsed with tap or distilled water(Garcia *et al*.,2018).

Purified of cryptosporidium oocysts

By using sugar flotation according to (Sawitri *et al*., 2020) as the following:-

The sample filtered through four layers of gauzes.Centrifuged at 550 g for 7 min. remove supernate and take the precipitation and taken one ml of the sample solutions was added to sugar solution in tube and mixed thoroughly; sugar solution was added again to the brim of the tubes as to form a convex surface at the mouth of the tubes. For the centrifugal floatation method, the mixed solutions were centrifuged at 550 g for 7 min Then sugar solution was added again up to the brim of the tubes same as the floatation method. Five minutes after, the samples were picked up from the surface, by Pasteur pipette and examined under a microscope.Washed the sedimentation 3 times with phosphate buffer saline and examined under light microscope.

Calculation oocysts of *cryptosporidium* parasite per 1ml

The oocytes count was done by using light microscope ×100then the total numbers of oocytes per 1ml calculate according to the following equation: Number of oocytes in 1 ml = (1000 x calculated oocytes number) / 8 (Al-Dahhan & Zghair,2020).and used oocytes concentration 1×106 that saved in phosphate buffer saline (PBS) al 4c̊.

Experimental infection

Used 40 BALB/C male mice with weight(to doing the experimental and exam the stool to ensure it clean from cryptosporidiosis . Divided the mice to five experimental groups as follow :-

Group A:- negative control , group B positive control that infected with cryptosporidium parasite did not exposure to copper nanoparticles , group C mice infected with cryptosporidium

oocysts exposure to copper nanoparticles at concentration (0.01) gm , group D infected with cryptosporidium oocysts exposure to copper nanoparticles with concentration (0.03) gm , group E infected with oocycsts exposure to copper nanoparticles with concentration (0.05) gm each group exposure to nanoparticles for 30 min and examined the feces of experimental mice daily until appear infection, calculate number of oocysts in each group.

Molecular identification of cryptosporidium

By using real- time PCR for detection *Cryptosporidium* ssp. based on **18Sr RNA** gene from feces samples .

Results and discussion

Table 1:-results of mice that take oocysts that exposure to CuNPs.

Cu nano. Concentration	No. infected mice	Mean oocysts counts/ infected mouse	Rate
Negative control	0/5	-	-
Positive Control	5/5	11104	100%
0.01	4/5	6313	56.84%
0.03	2/5	6875	61.91%
0.05	1/5	4000	36.02%

The results of using copper nanoparticles show upper result at concentration (0.03) gm. With rate of oocysts (61.91%) and low rate of oocysts at concentration (0.05)gm. With rate (36.02%) while the concentration(0.01)gm. give rate (56.84%). results of (0.03)gm. of copper oxidase nanoparticles may be explain according to to a hormetic effect. Hormesis is a dose-response phenomenon that describes growth stimulation at low doses and growth inhibition at high doses (Choi *et al.,* 2018). Hormesis is considered to be an adaptive response of biological systems to modest environmental challenges (Jiao *et al.,*2014). A hormetic response has been previously encountered with low concentrations of Ag+ ions and AgNPs, which enhanced the growth of E. coli (Xiu *et al.,* 2012; Fauss et al., 2014).

Conclusion

The dangerous biological of Cryptosporidium oocysts allowed these experiments to be conducted in real drinking water to investigate the impact of nanoparticles at concentrations close to their allowable limit in the water. Our results indicated that shorter contact times are recommended for better C. oocyst inactivation (30 min at0.01 gm , 0.03 gm, and 0.05 gm concentrations). Additionally, gaining of safe, effective, and cheap water disinfectant against *cryptosporidium* spp. contamination and perhaps against many other parasites and microbial ones is possible using nanotechnologies .

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