Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 2, 2021, Pages. 4335- 4342
Received 20 January 2021; Accepted 08 February 2021.

Scolicidal Effects of Silver – Copper (core-shell) Nanoparticles Against *Echinococcus granulosus* Protoscolices *in vitro*

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ABSTRUCT

The current study included the preparation and diagnosis of the Silver - Copper (core - shell) nanoparticlesand the nanoparticles on which the commercial drug Albendazole is loaded and the study of their effect on the Protoscolices of hydatid cyst disease *in vitro* , this is a comparison with the effect of the commercial drug alone with the presence of the control treatment that includes the addition of distilled water only **,** The results showed that the Silver-Copper (shell-core) nanoparticles had a very high effect on the vitality of the Protoscolices and this effect increased with increasing concentration and duration of exposurewith high significant differences but less than the intensity of the effect of the nanoparticles alone, the two compounds had a high and significant effect compared to the commercial drug when used alone**.**

Keywords : Protoscolices , Nanoparticles , Echinococcosis , Hydatid cyst.

INTRODUCTION

Echinococcosis is a zoonotic disease caused by the tapeworm of the genus *Echinococcus* . This parasite lives in the intestine of the final hosts in an adult form . In the intermediate host, the larval form is present as cysts within the internal organs and this disease is one of the biggest health problems common in some countries and has different forms, as cystic Echinococcosis is the most common and caused by *E. granulosus*because its larvae develop as single and separate cysts and it is less dangerous and more treatable than other forms such as *E. multilocularis* causing the disease. alveolar echinococcosis and *E.vogeli* that cause polycystic echinococcosis (CFSPH, 2020) .

According to Higuita*et al*. (2016) the squamous cyst parasite infects more than one million people around the world annually and is the cause of economic losses estimated at about three billion dollars each year.Hydatid cyst disease in developing countries such as countries in the Middle East remains a major health obstacle, and its highest prevalence rates are concentrated in rural areas where there are many sheep pastures, which represent the most important intermediate host Abdulhameed*et al*., 2019; Thys *et al*., 2019).

In Iraq, in particular, it is one of the main health problems (CDC, 2012), It is one of the endemic diseases in Iraq country and there are no organized national programs for disease control and surveillance (Athmar& Ban-Abbas, 2014). The most cases of human infection were recorded in the central and southern regions, including the governorates of Basra, DhiQar and Muthanna (Abdulhameed*et al*., 2019). It poses a health, social and economic dilemma due to the absence of

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an effective drug against it and the absence of pathological symptoms even after the disease reaches an advanced state, especially in the important organs such as the brain and heart, so it is difficult to control therapeutic and surgical in those organs, because of this risk generated attempts Solutions were used to reduce the disease and reduce its spread (Kharebov, 1997).

Some studies have shown that nanoparticles of silver AgNps, gold AuNps, chitosan and other metal oxides have an inhibitory or toxic effect for many parasites such as *Giardia*, *Leishmania*, *Toxoplasma*, *Plasmodium* and some insect larvae (Elmi*et al*., 2013). The nanoparticles of silver made from nanoparticles are the most effective due to their direct contact with the environment and their anti-parasite, bacterial and viral effects (Mahmoud*et al*., 2014). Therefore, it is recommended to use nanoparticles to get rid of parasites as they are effective and less harmful drugs as well as an effective vaccine for controlling parasites (Elmi*et al*., 2013).

MATERIAL AND METHODS

Collection of hydatid Cysts and Protoscolices

Sheep livers naturally infected with hydatid cysts were collected from locally stores , Karbala city , Iraq placed in clean containers and transferred to the Parasitology Lab.- Department of Medical Laboratory Techniques / Al-Safwa University College, the livers were washed with running water to get rid of blood and impurities, then their surfaces were sterilized with dilute ethyl alcohol at a concentration of 70% and at laboratory temperature the fluid of the cysts was withdrawn by sterile medical syringes 5 ml and by means of clean and sterile scissors the cyst was opened.Phosphate Buffer Saline Solution(PBS) was added to the washing bottle and antibiotics were added to the buffer (Crystalline penicillin at 2000 units / liter and Streptomycin at 1 g / L).Where the germ layer was washed to remove the suspended protoscolices in it, the liquid was left for a period of time for all the protoscolices to be deposited , after then were collected and deposited and the float was removed and a little of what was left of it was placed in plastic test tubes for sedimentation in the Centrifuge machine, where it was deposited for 15 minutes 3000 rpm/m.(Smyth, 1985) .

Preparation of Nanoparticles

The Silver-Copper (shell-core) nanoparticles was preparedaccording to (Nadagouda&Varma, 2007) . 10 mL (0.1 N) of ascorbic acid (vitamin C) was allowed to interact with 2 mL (0.1) N of calcium chloride CuCl2 which act as the core , then added to the shell which is about 2 ml of (0.01 N) AgNo3and left to interact with shaking at room temperature for an hour to form a gray precipitatethat was separated from the filtrate and washed with distilled water and spread it until drythen grinded and collected in tubes glass and kept at room temperature until use.

Preparation of commercial drug

The drug was obtained from a commercial pharmacy and it is Albendazole 200 mg tablets, manufactured by Julphar Company - United Arab Emirates. The tablets were crushed and the drug was used as a control treatment for comparison with the nanoparticles .

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Loading the commercial drug on NPs

250 mg of the nanoparticles which preparedPreviously was mixed with 250 mg of the commercial drug in 25 ml of distilled water, the mixture was left for 12 hours on the magnetic stirrer and then left to precipitate and the filtrate was separated from the sediment and the sediment was washed with distilled water and left to dry, after complete drying, the sediment was collected , grinding and kept in a glass container at room temperature until use.

Diagnosis of NPs

The prepared nanoparticles were diagnosed to ensure that they carry the nanoscale properties by Fourier-transform infrared spectroscopy (FTIR), Atomic force microscopy (AFM) and X-ray diffraction (XRD), and the shell-core characteristic between the two compounds was confirmed by Scanning electron microscope (SEM) and examined at Al-Fadhelfoundation / Babel - Al-Hillah branch, SEM and XRD examination were carried out in Iran.

The efficacy of Nanoparticles and Albendazole drug in the viability of Protoscolices*in vitro*

Concentrations of 50, 125, 250 and 500 micrograms / ml were used. 0.5 ml of the protoscolices suspension was placed in 10 ml test tubes and then the Nanoparticles was added after the stock solution was prepared. Three replications were made for each of the four concentrations of the drug and the Nanoparticles with The presence of a control treatment added to it with distilled water only. Protoscolices vitality were examined after 10, 30 and 60 minutes of addition at 37 °C. Protoscolices viability was examined by taking 10 µL of the suspension and adding the dye and examining under a microscope where the living heads were stained green and dead heads were stained red (Nematollahi*et al*., 2018(.

RESULTS AND DISCUSSION

Effect of Albendazole drug in the viability of Protoscolices

The results showed according to Table 1, that the effect of treatment with a concentration of 50 mg / ml had no significant effect after 10 minutes of treatment, and the situation continued after 30 minutes and after 60 minutes of treatment , compared with the control treatment .The significant differences began to appear with increasing concentration, but these significant differences were insignificant compared with the control treatment when using concentrate 125mg / ml and after 10 minutes, and these slight differences continued with increasing time up to 60 minutes.

Significant differences increased at concentration 250 mg / ml compared to control treatment, but the increase in the time period of exposure to treatment did not affect in the vitality of protoscolices .The greatest value of the significant differences appeared with a concentration of 500 mg / ml compared with the control treatment, but there was no clear significant difference with the time increase when the protoscoliceswere exposed to this concentration.

When comparing the concentrations, we will find that the effect of the treatment increased with the increase in concentration compared to the control treatment, as the concentration 50 mg / ml

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had no effect on the vitality of the substrates, while this effect increased with the concentration of 125 mg / ml, reaching a maximum of 500 mg / ml. With high significant differences.As for time, it did not have a clear effect on vitality, and there were no significant differences . These results were in agreement with Al-hamiary(2010) and Al-Nakeeb (2004) who indicated that the treatment of Albendazole had an effect on primordial viability that increased with increasing concentration.

As this drug is considered one of the best drugs used in parasite resistance, the principle of its action depends on binding with the B-tubulin protein, causing the polymerization process to be canceled in the microtubules of this protein causing a group of damage to the parasite cells from the transfer, growth and obstruction to the absorption of glucose in worms and parasitic larvae ,this leads to the depletion of the stock of animal starch (Glycogen), thus reducing energy production ATP until the parasite dies (Karakay , 2007) .

Table (1) : Effect of Albendazole drug in the viability of Protoscolices

Treatment	Time / Conc.	10 M.		30M.		60M.		SIG.
		Mean	SD	Mean	SD	Mean	SD	
Albndazole	control	37.67	2.08	36.00	14.93	35.00	5.00	0.654
	50	31.00	12.12	26.67	0.58	25.67	1.53	
	125	24.00	1.73	21.67	2.89	21.00	1.73	
	250	18.33	1.53	17.00	1.00	15.00	1.73	
	500	12.67	0.58	12.00	1.73	10.33	0.58	
	LSD	10.194		12.490		4.721		
	Sig.	0.002		0.014		0.0001		

Effect of Silver – Copper (core –shell) Nps in the viability of Protoscolices

The results showed that the silver-copper nanoparticles (core-shell) had a significant effect on the vitality of the substrates compared to the treatment of Albendazole, as this compound had a significant effect even with the lowest concentration of 50 mg / ml, where significant differences appeared compared to the control treatment, and these significant differences increased. By increasing the concentration significantly and clearly, it reached its peak concentration at 500 mg / ml, where the colic killing rate was 100%.The increase in the exposure period also had an effect on the vitality of the Protoscolices as well with very large significant differenceseven with the lowest concentration of 50 mg / ml, reaching a peak at 60 minutes, and with the concentration of 125 mg / ml, the nanoparticles had an effect at 10 minutes which increased dramatically with increasing time and significant differences.As for the concentration of 250 and 500 mg / ml, their effect was the greatest and clear since the first ten minutes of exposure, with very large significant differences.

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As shown in Table 2, the two concentrations 250 and 500 mg / ml are the most effective in the vitality of the protoscolices , compared with the lowest concentrations of 50 and 125 mg / ml .The results are in agreement with Norouzi*et al.* (2020) who showed that silver nanoparticles have a scolicidaleffect, and this effect increases with increasing concentration and increasing the exposure time, as is the case with copper nanoparticles, but with less effect than silver .

The nanoparticle oxides, including the silver nanoparticles have heterogeneity in surface propertiesbandgap causing a high increase in detonation that affects the surface adsorption process and thus their penetration into the surfaces (Immanuel*et al.*, 2019) .

Table (2) : Effect of Silver – Copper (core –shell) Nps in the viability of Protoscolices

Treatment	Time / Conc.	10 M.		30 M.		60 M.		SIG.
		Mean	SD	Mean	SD	Mean	SD	
Silver – Copper (core –shell) Nps	Control	37.67	2.08	36.00	14.93	35.00	5.00	0.312
	50	27.33	2.31	20.33	5.51	0.00	0.00	
	125	14.00	7.00	0.00	0.00	0.00	0.00	
	250	0.00	0.00	0.00	0.00	0.00	0.00	
	500	0.00	0.00	0.00	0.00	0.00	0.00	
	LSD	6.232		12.950		4.068		
	Sig.	0.0001		0.0001		0.0001		

Effect of Drug loading on Silver - Copper (shell – core) nanoparticles in the viability of Protoscolices

The drug loading on the silver-copper nanoparticles (core-shell) had a clear effect on the vitality of the protoscolicescompared to the control treatment and even with the lowest concentration usedwhich was 50 mg / ml, where there were clear significant differences in the effect and this effect continued to increase with increasing concentrations until the significant differences became very high with a concentration of 500 mg / ml, as show in table 3 .The duration of exposure also had an effect on the vitality of the protoscolices as well as the results showed that the viability of the protoscolices decreases with the increase in the duration of exposure compared to the control treatment. Results were similar with higher concentrations 125, 250 and 500 mg / ml and with greater significant differences, the viability reached zero after 30 minutes of exposure time with concentrations of 250 and 500 mg / ml.These results agreed withTorabi*et al.* (2018) who used the drugs Albendazole and Praziquantel loaded with chitosan nanoparticles in the treatment of microcysts of *E.granulosusin vitro*and found it very effective .

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It also agreed with Bakhtiar*et al.* (2019) that showed that Albendazole loaded on selenium nanoparticles at concentrations of 250 and 500 mg / ml has an effect of 100% in killing protoscolices after exposure for 5 days .

Table (3) : Effect of Drug loading on Silver - Copper (shell – core) nanoparticles in the viability of Protoscolices

Treatment	Time / Conc.	M.10		M. 30		M. 60		SIG.
		Mean	SD	Mean	SD	Mean	SD	
Drugloading on Silver - Copper (shell – core) nanoparticles	control	37.67	2.08	36.00	14.93	35.00	5.00	0.653
	50	15.33	6.43	14.33	5.13	0.00	0.00	
	125	3.00	1.00	0.67	0.58	0.00	0.00	
	250	2.67	0.58	0.00	0.00	0.00	0.00	
	500	1.33	1.15	0.00	0.00	0.00	0.00	
	LSD	5.656		12.856		4.068		
	Sig.	0.0001		0.0001		0.0001		

According to the results, the commercial drug loaded on the nanoparticles is more effective in reducing the vitality of the protoscolices which was agreed with Bakhtiar*et al.* (2019) who indicated that Albendazole loaded on the selenium nanoparticles kills 100% of the protoscolices in 5 days compared to the drug alone, which kills 100% after 7 days .

CONCLUSION

The current research has found that the commercial drug becomes effective in eliminating the protoscolicesof hydatid cyst*in vitro* when it is loaded onto the Silver-Copper nanoparticles (core-shell) compared to the drug alone, and the nanoparticles alone is better than the commercial drug and is also betterfrom the drug loaded on the nanoparticles .

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