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Influence of cadmium and zinc on the viability of *Echinococcus granulosus* in Vitro

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Abstract

Fifty-two viable E. granulosus hydatid cysts were obtained from the livers of eleven cattle in Al-Qadisiyah province slaughterhouses between April and August 2024; then protoscoleces were dropped and treated with three different concentrations of cadmium and zinc (100 µg 1-1, 1000 µg 1-1, and 10,000 µg 1-1 metal ions) and a mixture of cadmium and zinc at a concentration of 10000 µg 1-1 and in vitro. This study found that these metals effectively kill protoscoleces, and Protoscoleces' activity reduced with increasing concentration and length of exposure compared to the control group. The results also revealed that the combination of cadmium and zinc had the largest effect on protoscolces viability than Albendazole, so cadmium and zinc; this could be attributed to the combined toxic effect of the two heavy metals.

***Keywords**: CE;* cadmium*;* zinc; *hydatid cysts*.

Introduction

Hydatid disease, also known as cystic echinococcosis (CE), is a global zoonotic disease. The larval stage of E. granulosus, which is present in intermediate hosts such as sheep, goats, cattle, and pigs, is the causative agent of CE (1-3). The adult stage is characterized by the presence of small tapeworms of E. granulosus in the small intestine of the definitive host, which is a carnivore that is responsible for the dissemination of eggs in the environment (1- 3). The CE disease threatens human health by forming parasitic tumors in the lungs, liver, brain, and other organs that could be fatal without proper treatment (4,5).

The infection of the definitive host begins when the viable protoscoleces of infected domestic intermediate hosts are eaten. This can happen when contaminated offal is fed to pets after a home slaughter, when abattoirs and slaughterhouses are not managed properly, or when stray dogs eat dead animals that have been left on the pasture (6-8). By eating the intermediate hosts or people, the eggs hatch in the small intestine and release six-hooked oncospheres (embryos) that go through the digestive wall and move through the bloodstream to different organs, mainly the liver and lungs (9,10). Within the organs, the oncosphere turns into a single-locus hydatid cyst that gets bigger over time and creates daughter cysts or protoscoleces that fill the inside of the cyst. When cysts burst, the protoscoleces are released and have the potential to form additional cysts in different locations inside the body (11-13). Major reasons for the high prevalence of echinococcosis are thought to be uncontrolled slaughter, insufficient abattoir/slaughterhouse facilities, and abundance of stray dogs (14,15).

In the germinal layer of viable hydatid cysts, protoscoleces are made. These are the infectious

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forms of the parasite for the final host. For unspecified causes, certain hydatid cysts are sterile and incapable of producing protoscoleces (16,17). Hydatid cysts are classified into two categories: fertile and sterile cysts, with the latter not producing protoscoleces, hence terminating the parasite's life cycle (18,19).

Heavy metals are the most toxic and harmful metals among the other elements that naturally occur in different concentrations in all ecosystems. Both zinc and cadmium belong to Group 12 of the periodic table and are closely related metals. Both ions can attach to the same macromolecular structures via nitrogen, oxygen, and sulfur (20-22). This is because their sizes and electron configurations are quite similar. On the other hand, cadmium is a non-essential metal that is not known to have any biological function in metazoans, in contrast to zinc, which is an essential element for all forms of life and has an important role in metabolic processes (23,24).

Parasites safeguard their hosts' tissues from the harmful effects of heavy metals by sequestering these substances within their bodies, thereby serving as environmental bio-indicators (25). demonstrated the susceptibility of parasites to cadmium. As cadmium levels in the host's intestine increased, the prevalence of parasites diminished to none. when the accumulated cadmium concentration exceeded 0.05 mg/kg, the threshold for meat (apart from offal) of cattle, sheep, pigs, and poultry as specified by Regulation (EU) No. 488/2014 amending Regulation (EC) No. 1881/2006, no parasites were found in the gut. Consequently, cadmium levels beneath the specified threshold affected parasite biodiversity (26). On the contrary found levels of some heavy metals (Cd, Al, Hg, and Pb) were found in parasitized cow and sheep liver with Echinococcus samples. In contrast, Echinococcus hydatid cysts isolated from cow and sheep livers yielded larger quantities of various metals than their uninfected counterparts. Moreover, the result of (27,28) indicated substantial disparities in the concentrations of most heavy metals between the fluids of sterile and fertile cysts. Heavy metal contents in hydatid cyst fluid obtained from the liver are markedly elevated compared to those in hydatid cyst fluid derived from the lungs (29,30). The study comes to the conclusion that these metals may make hydatid cysts sterile by changing the activity of heat shock proteins or causing cellular stress by making reactive oxygen species.

This study aimed to find out how harmful cadmium, zinc, and a combination of cadmium and zinc are to *E. granulosus* protoscoleces cells grown in a lab, comparing their effects to the treatment drug albendazole. This is a contributing factor to cellular stress and apoptosis, leading to the sterilization of *E. granulosus* hydatid cysts.

Materials and Methods

A. Sample collection

Fifty-two viable E. granulosus hydatid cysts were obtained from the livers of eleven cattle in Al-Qadisiyah province slaughterhouses between April and August 2024. 70% ethyl alcohol was applied to the hydatid cysts in order to sterilize their surface, then hydatid liquid was withdrawn with a sterile needle and collected in tubes for separation by centrifugation. The protoscoleces deposits at the bottom of the cylinders were washed three times with phosphate-buffered saline solution (PBS). They were then stained with 0.1% eosin to see which protoscoleces were viable and which ones were not. In contrast, those not stained with eosin were considered viable according to the convention (31).

B. Estimation of protoscoleces viability

Aqueous eosin stain 0.1% was used by adding a drop of this stain to an equal volume of protoscoleces suspension using a micropipette, agitating the solution thoroughly, followed by obtaining a drop for microscopic examination at 20x magnification. The percentage of live protoplasts that appeared green was calculated, while the dead ones appeared

red due to the penetration of the aqueous eosin pigment through their walls (31).

C. Protoscoleces counting

Protoscoleces were counted according to (32). approach. After testing protoscoleces, mix protoscoleces with phosphate-buffered saline (PBS) dispersion, and use a 10 µL micropipette to transfer the exact amount. The count was performed using a microscope, and this process was reiterated three times. The following formula was used to count the viable protoscoleces in a 1 ml sample:

Viability in 1 ml = number of protoscoleces in (10 µl) x 100

D. Preparation of heavy metals solutions

Solutions of heavy metals were prepared according to (33) as follows :

Stock solutions containing 100 ppm cadmium and zinc were prepared individually by dissolving 20.84 mg of cadmium chloride (CdCl2.5/2H2O) with a molecular weight of 136.28 in 100 ml of water. Zinc chloride (219.304 molecular weight) at 19.5 mg was taken and dissolved in 100 ml of distilled water to ensure that the appropriate concentration of metal ions was achieved.

2-The addition of distilled water to stock solutions allowed us to obtain concentrations of metal ions from cadmium, zinc, and a combination of cadmium and zinc at 100 g/L, 1000 g/L, and 10,000 g/L (33).

E. Influence of zinc, cadmium, and mixtures of zinc and cadmium on protoscoleces viability of E. granulosus

1- 1 ml of either zinc chloride (ZnCl2) or cadmium chloride (CdCl2.5/2H2O) to test tubes that already had Krebs Ringer solution and protoscoleces liquid in a 4:1 ratio. About 2000 protoscoleces were present in each tube.

2- Added 1 mL of albendazole at that concentration (10 mg/mL). The drug was in the form of an emulsion in test tubes containing both protoscoleces liquid and Krebs Ringer solution at a ratio of 4:1 to achieve the best possible treatment.

3- Utilize a designated volume of the preserving medium and incorporate protoscoleces; it served as a control group.

4- Shake the tube well and take 10 microliters of protoscoleces from each tube, with 10 microliters of eosin stain. Vitality of protoscoleces was observed over time periods (15, 30, and 60 min) and with three repetitions for each concentration. The percentage of life and protoscoleces that perished was calculated (dead protoscoleces were identified by their color red, whereas live protoscoleces were identified by their color bright green)

5- The current study's data were statistically analyzed using the Statistical Package for the Social Sciences (SPSS) program. The data were described. test the significance level of (0.05>P).

Result

A. Influence of zinc, cadmium, and a mixture of zinc-cadmium on protoscoleces viability of E. granulosus

Table 1 and Figure 1 show that the number of viable protoscoleces significantly reduced compared with the control group ($p < 0.05$) with increasing concentration and duration of exposure to zinc and cadmium. Both zinc and cadmium at 100 mg/l caused a decrease in the viability rate of protoscoleces from 66.6± 0.88, 39.6± 4.84 respectively at the 15th minute of the experiment to 36.3± 0.88, 25± 2.88 respectively at the 60th minute of the experiment, At a concentration of 10,000 mg/l, the viability rate of protoscoleces diminished from 41±0.57 and 34±1.52 at the 15th minute to 22.33±0.88 and 14.33±1.45 at the 60th minute of the experiment. The results showed that starting in the 15th minute of the experiment, cadmium

significantly killed more protoscoleces than zinc did compared to the control group ($p < 0.05$). The mean of viable protoscoleces ranged between 31.2±2.90 - 23.22±8.85 for cadmium. The range of mean viable protoscoleces ranged between 51.8±4.41 and 31.88±2.74.

The results also showed that treating the protoscoleces with a mixture of cadmium and zinc was significantly superior to treating them with cadmium and zinc separately. The mean of viable protoscoleces was 14.8±1.65 for treating them with a mixture of cadmium and zinc, while the lowest mean of viable protoscoleces was 23.22±8.85, 31.88±2.74 for treating them with cadmium and zinc separately. The number of viable protoscoleces significantly decreased compared with the control group ($p < 0.05$). With increasing duration of exposure to a mixture of cadmium and zinc, the viability rate of protoscoleces decreased from 21±0.57 at 15 minutes of the experiment to 10±0.57 at the 60th minute of the experiment.

B. Influence of albendazole and heavy metals on protoscoleces viability

Table 2 and Figure 2 showed that the number of viable protoscoleces significantly decreased compared with the control group ($p < 0.05$) with increasing duration of treatment with albendazole. Albendazole at 10 mg/l caused a decrease in the viability rate of protoscoleces from 81±0.57 at the 15th minute of the experiment to 61.6±4.91 at the 60th minute of the experiment.

The results also showed that treating the protoscoleces with cadmium, zinc, and a mixture of cadmium - zinc was significantly superior ($p < 0.05$) to treating them with albendazole, at the 60^{th} minute the mean of viable protoscoleces was 61.6±4.91 for treating them with albendazole while the lowest mean of viable protoscoleces was 20±1.07, 34.3±1.98 and 10±0.57 respectively for treating them with cadmium, zinc, and mixture of cadmium - zinc separately .

The results also showed that the mixture of cadmium-zinc was significantly superior ($p < 0.05$) in reducing the vitality of protoscoleces over all other treatments in all treatment periods.

Table 1: Influence of zinc, cadmium, and a mixture of zinc-cadmium on protoscoleces mortality of E. *granulosus*

Concentration	Zn				Cd			
	15 min.	30 min.	60 min.	Mean	15 min.	30 min.	60 min.	Mean
100 mg/l	66.6± 0.88	52.6± 1.2	36.3± 0.88	51.8± 4.41	39.6± 4.84	29± 3.51	25± 2.88	31.2± 2.90
1000 mg/l	59.6± 0.88	46.6± 0.88	32.3± 1.45	46.2± 3.98	35± 0.57	24.3± 0.33	15± 0.57	24.7± 2.9
10000 mg/l	41±0.57	32.33±1.45	22.33±0.88	31.88±2.74	34±1.52	21.33±0.88	14.33±1.45	23.22± 8.85
Zn 10000 + Cd 10000 µg/l	21± 0.57	13.6± 0.88	10± 0.57	14.8± 1.65	21± 0.57	13.6± 0.88	10± 0.57	14.8± 1.65
Control	874.6 ±4.33	872.6±4.05	869.3±4.63	874.6±4.33	813± 4.16	809.6± 4.04	815± 4.72	812.5± 2.35
LSD(P<0.05)	14.08			9.72	13.58			8.24

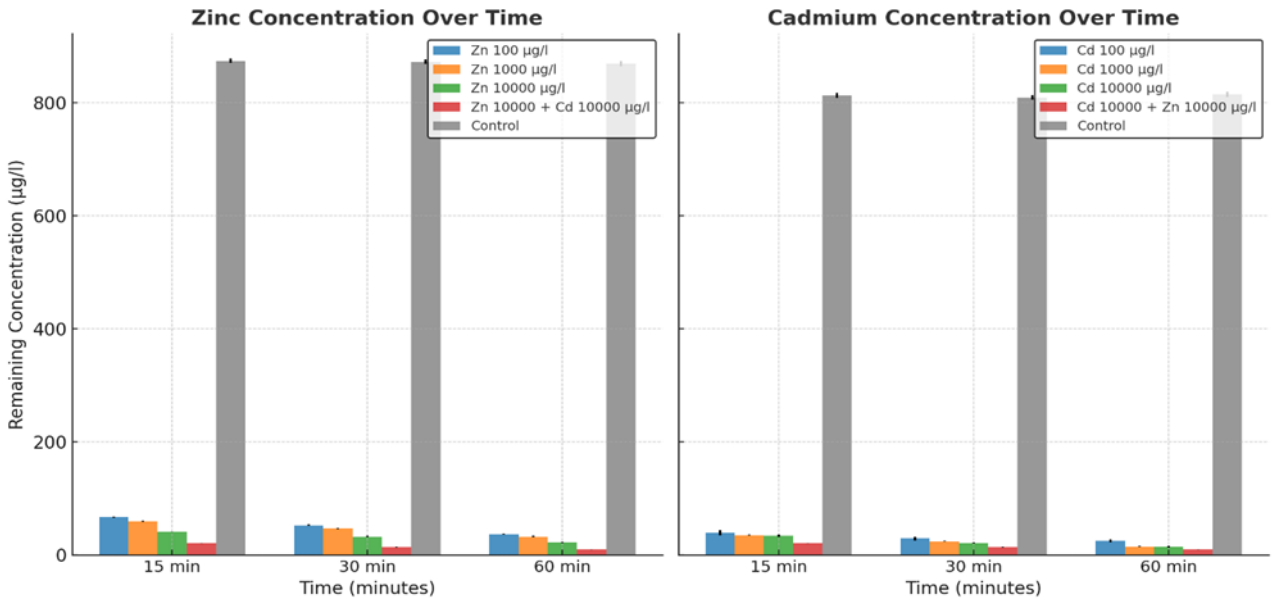

Figure 1: Influence of zinc, cadmium, and mixture on protoscoleces mortality of E. granulosus

Table (2): Comparison of Bendazole and metals on viability protoscoleces of *E. granulosus*

Substance	Time		
	15 min.	30 min.	60 min.
Zn	63.1±3.15	49.6±2.01	34.3±1.98
Cd	37.3±2.13	26.65±1.21	20±1.07
Zn10000 + Cd 10000 µg/l	21±0.57	13.6±0.88	10±0.57
Bendazole 10 mg/l	81±0.57	75.3±0.88	61.6±4.91
Control	874.6±4.33	872.6±4.05	869.3±4.63
LSD(P<0.05)	**8.12**	**8.56**	**9.03**

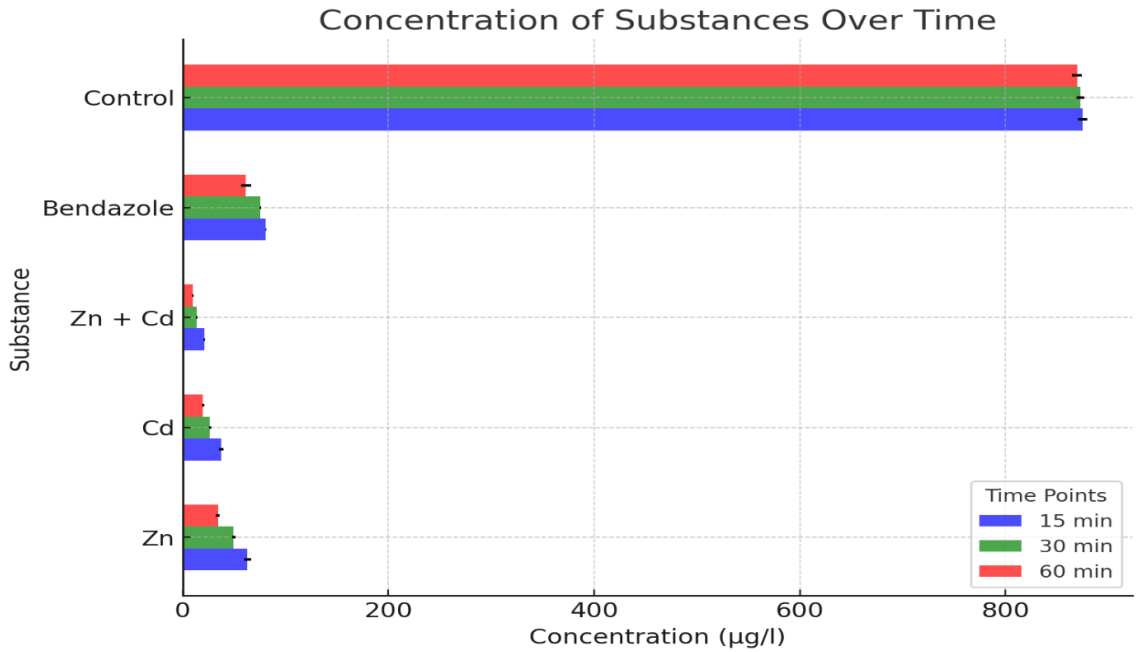

Figure 2: Comparison of Bendazole and metals on viability protoscoleces of E. granulosus.

Discussion

The eosin dye penetration effect was used to test the viability of protoscoleces without using any outside inversion or movement markers. When looking at protoscoleces under a microscope, it can be hard to tell the difference between ones that are moving and ones that are not. This method helps us avoid the mistakes that happen when we count them based on how they move back and forth. Additionally, some protoscoleces may not be able to invert for unidentified physiological reasons, leading to them being mistakenly marked as dead. This is another reason why the external inversion method frequently produces poor results (34,35). While viable protoscoleces maintain their natural color, eosin dye penetration is a physical process that is connected to the plasma membrane's permeability. When a

physiological defect arises, permeability increases, allowing the dye to infiltrate (36,37).

From the current results, it appears that Albendazole, cadmium, zinc, and the mixture of cadmium and zinc have a clear effect in reducing the viability of the protoscoleces, and this effect is directly proportional to the duration of preservation and the increase in concentration of the protoscoleces. According to (25,26), who discovered that sterile hydatid cysts contain high concentrations of heavy metals, treating the parasite in vitro with the aforementioned elements separately resulted in a decrease in the mean rate of viability of the protoscoleces. This may be a result of the generation of reactive oxygen species (ROS) or their impact on the action of heat shock proteins, which may stimulate cellular stress. (38).

The current study also found that cadmium killed the protoscoleces more quickly and effectively than zinc. It may be because cadmium binds to mitochondria, changes the DNA repair process, triggers apoptosis, and releases reactive oxygen species, all of which inhibit cell growth. At low concentrations, cadmium has a potent ability to inhibit cellular respiration (39,40). Additionally, the results demonstrated that the mixture of zinc and cadmium killed more protoscoleces than either albendazole, cadmium, or zinc by itself. That might be because the two heavy metals are toxic when they work together (41,42).

Conclusion:

Environmental factors influence the survivability of protoscolces and the fertility of hydatid cysts, and the complete life cycle of E. granulosus. Heavy metals can induce apoptosis by generating reactive oxygen species (ROS). This may be the primary reason hydatid cysts are incapable of reproduction.

Conflict of interest: NIL

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