



Research Article

Heavy metals in the hydatid fluid and their effect on the fertility of *Echinococcus granulosus*

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Abstract

Heavy metals have been recognized as risk factor for living organisms. This study aims to evaluate the effects of some heavy metals including Cadmium, Cobalt, Lead, and Zinc on the fertility of hydatid cysts of *Echinococcus granulosus* isolated from the livers and lungs of cattle aged 2 to, more than 4 years, in Thi- Qar governorate. Fifty-five hydatid cysts (fertile & sterile) were isolated from the livers and lungs of thirty-three cattle. Flame Atomic Absorption Spectrophotometer (FAAS), was used to assess the concentration of heavy metals. The result data showed that there are significant differences, in most of the heavy metals in the fluid of sterile and fertile cysts. Heavy metal concentrations in hydatid cysts fluid extracted from the livers are significantly higher than their concentration in hydatid cysts fluid extracted from the lungs. This study concludes that these metals may represent a cause of hydatid cysts sterility through stimulating cellular stress by generating Reactive Oxygen Species or through their effect on the action of heat shock proteins.

Keywords: *E. granulosus*, Hydatid cysts, Sterile, Fertile, Heavy metals

Introduction

The larval stage of *Echinococcus granulosus* is a causative agent of cystic echinococcosis (CE), a zoonotic disease worldwide (Thompson, 2017). The adult stage of *E. granulosus* is found in the small intestine of the definitive host, a carnivore that is responsible for egg dissemination in the environment (Romig, 2017).

The parasite's infective form to the definitive host is produced in the germinal layer of fertile hydatid cysts. For unknown reasons, some of the hydatid cysts are sterile and unable to generate protoscolices (Vatankhah et al., 2003; Cabrera et al., 2008). So, the hydatid cysts are divided into two types: fertile and sterile cysts that do not generate protoscolices, therefore ending the parasite's life cycle (Daryani et al., 2009).

Previous studies suggested different causes for hydatid cyst sterility such as: may Hydatid cysts failure to form protoscolices as a result of calcification, infection with bacteria, (Markell et al., 1986; WHO, 1996) as indicated by

Sharif et al. (2005) that some enzymes may contribute to the sterility of hydatid cysts. Manterola et al. (2006) added another reason is that fertility is associated with the type of cyst, which was observed high ratio of fertility in multivesicular compared with univesicular cysts.

Paredes et al. (2007) conclude that apoptosis may be involved in hydatid cyst sterility, The presence of parasitic heat shock protein and annexin A13 exclusively in sterile cysts, as well as an increased spectral number of Cathepsin B., while, Cabrera et al. (2008) mention the oxidative damage to mRNA is part of the mechanism that induces infertility.

Aziz et al. (2011) explained the presence of Heat Shock Proteins (HSP), Annexin A13, and Cathepsin B in the hydatid fluid supports the hypothesis of cellular stress and apoptosis as the cause of hydatid cyst sterility. While Saadoon et al. (2014) reported that the oxidative stress resulting from the increase in the level of free radicals, which results from the immune mechanism of the host against echinococcosis infection, played a significant role in affecting the fertility of cysts, and

found that the level of antioxidants in fertile hydatid cysts is higher than in sterile cysts. Immunological factors (IgG1), as well as oxidative DNA damage caused by internal environmental factors, have been suggested to play a role in hydatid cyst sterility (Cabrera et al., 2008; Rieszle et al., 2014). While Fallah et al. (2021) referred to the host likely playing a key role in hydatid cyst fertility. In addition to that, Farhood et al. (2022) confirmed that no correlation between the haplotype of the parasite and the sterility of hydatid cysts, and suggested, maybe that environmental factors might play a key role in the fertility of hydatid cysts.

Several findings on *E granulosus* have been conducted globally and locally, including; Andresiuk et al. (2013); Romig et al. (2015); Al-Saadi et al. (2021) and Al-Atabi (2022). Most of the studies were focused on identifying its strains.

The goal of this study was to see if heavy metals play a role in modulating protoscolices development at the germinal layer of *E. granulosus* hydatid cysts, which is one of the causes of cellular stress and apoptosis, resulting in *E. granulosus* hydatid cyst sterilization, which was achieved by measuring the concentration of several heavy metals (Cd, Co, Pb, and Zn) in sterile vs. fertile cysts.

Materials and Methods

Samples Collection

Fifty -five fresh fertile and sterile *E. granulosus* hydatid cysts were obtained from the livers and lungs of thirty-three cattle in Thi-Qar province slaughterhouses, during the period from January to July 2021. These samples included, 33 hydatid cysts from livers (18 sterile and 15 fertile) and 22 hydatid cysts from lungs (7 sterile and 15 fertile).

Fertility determination of hydatid cysts

Cysts were processed as described by Smyth & Barrett (1980) and Macpherson (1985). Fertile cysts were identified under a light microscope by the presence of grown protoscolices attached to the germinal layer, and free protoscolices in the hydatid fluid. The sterility cysts were those showing the absence of the protoscolices by microscopic observations.

Extraction of heavy metals from hydatid cysts

The hydatid liquid was digested according to Ji & Ren, (2002) with some modification. In a Pyrex 10 beaker, 2 ml of 70% nitric acid and 1 ml of 70% perchloric acid were mixed with 0.5 ml of the hydatid liquid filtrate (after centrifuging the hydatid liquid at 3000 rpm/min for 20 minutes). The produced mixture was heated on a hot plate at 100°C, even before drying. The volume was ultimately completed to 10 ml by 30 % hydrochloric acid, which was then heated again on a hot plate at 100 °C even before drying, the residue dissolved in 3 ml distilled water, and the volume was finally completed to 10

mL by distilled water. It was then put into a test tube to be ready for measurement by FAAS.

Estimation of heavy metals

Flame atomic absorption spectrometry was used to determine heavy elements (Cd, Co, Pb, and Zn) (FAAS-Pg. United kingdom-UK). The technique was followed exactly as specified by the manufacturer.

Statistical Analysis

Statistical analysis was performed using SPSS version 23 software. T-tests were used to compare the concentrations of heavy metals in sterile and fertile cysts in the different groups studied, and standard deviation values were extracted; P-values were used as significance levels at P≤ 0.05, according to Field (2012).

Results

The study samples were divided into two groups based on the sterility of the cysts. There is a significant difference in the concentration of all heavy metals when comparing the fluid of sterile and fertile cysts at P≤ 0.05. In addition, a significant difference was shown in the concentration of all heavy metals between the fluid of sterile cysts and fertile cysts of livers and lungs hydatid cysts (P≤ 0.05) (Table 1 and 2).

Table 1: concentration of heavy metals ug/L in hydatid cysts fluid isolates from both fertile and sterile cysts, for cattle livers, Mean ± SD

N	The element	Sterile cysts	Fertile cysts	Significant difference
1	Cd	19.05 ±81.75	3.05±26.75	0.000
2	Co	6.56 ±43.46	7.58±34.22	0.001
3	Pb	11.94 ±75.15	4.49±27.98	0.000
4	Zn	14.43 ±88.52	2.85±47.66	0.000

Table 2: concentration of heavy metals ug/L in hydatid cysts fluid isolates from both fertile and sterile cysts, for cattle lungs, Mean ± SD

N	The element	Sterile cysts	Fertile cysts	Significant difference
1	Cd	8.74 ±72.38	4.79±20.24	0.000
2	Co	11.6 ±48.46	4.56±37.71	0.005
3	Pb	15.95 ±68.1	4.02±22.61	0.000
4	Zn	10.06 ±61.39	3.58±45.99	0.000

This result showed a significant difference in concentration of heavy metals when comparing hydatid cyst fluid isolated from both sterile cysts in the livers and fertility in lungs hydatid cysts (p ≤ 0.05) (Table 3).

Table 3: concentration of heavy metals ug/L in hydatid cysts fluid isolates from both sterile in livers, and fertile in lung cysts for cattle, Mean ± SD

N	The element	Sterile cysts	Fertile cysts	Significant difference
1	Cd	14.02 ±84.02	2.75 ±16.62	0.000
2	Co	3.52 ±45.54	5.87±38.17	0.015
3	Pb	10.37 ±78.06	1.89±19.84	0.000
4	Zn	14.61 ±90.13	2.74±47.16	0.000

The concentration of Zinc showed a significant difference between the fluid of hydatid cysts isolated from both sterile livers and lungs. In contrast, the other heavy metals showed no significant difference between the two groups in the same cattle (Table 4). In addition, there is no significant difference in heavy metals concentration between hydatid cysts fluid isolated from both fertile cysts isolated from livers and lungs (Table 5).

Table 4: concentration of heavy metals ug/L in hydatid cysts fluid isolated from both sterile cysts isolated from livers, and lungs in the same organism for cattle Mean± SD

N	The element	Sterile cysts	Fertile cysts	Significant difference
1	Cd	22.80 ±86.93	8.74 ±72.38	0.114
2	Co	11.61 ±48.56	8.68±41.56	0.199
3	Pb	10.88 ±78.49	15.95±68.10	0.150
4	Zn	15.33 ±91.62	10.06±61.39	0.000

Table 5: concentration of heavy metals ug/L in hydatid cysts fluid isolated from both fertile cysts isolated from livers, and lungs in the same organism for cattle, Mean ± SD

N	The element	Sterile cysts	Fertile cysts	Significant difference
1	Cd	3.16 ±27.35	3.39 ±23.87	0.070
2	Co	6.90 ±39.61	3.19±37.24	0.425
3	Pb	4.41 ±28.74	3.70±25.37	0.147
4	Zn	2.49 ±47.16	4.13±44.81	0.221

Discussion

The concentrations of heavy metals cadmium, cobalt, lead, and zinc was recorded in the current study. They were measured from the hydatid fluid of sterile hydatid cysts of cattle livers and were higher compared with their concentrations in hydatid fluid of fertile cysts isolated from livers (Table 1). Similarly, their concentrations in the fluid hydatid of sterile cysts hydatids of cattle lungs were high concentrations compared to their concentrations in hydatid fluid of fertile cysts of cattle lungs (Table 2). The same trend of heavy metals concentrations was detected in mix infection animals fertile /sterile in both the liver and lung respectively

(Table 3), Perhaps because the liver, as the main metabolic organ, accumulated the highest concentrations of metals from other body organs (Hogstrand & Haux, 1991), or, this may be owing to the low-fat content of lung tissues, as the concentrations of elements are lower, in tissues with low-fat content (Vinodhini & Narayanan, 2008).

The exceed heavy metals investigated in this study are non-essential, toxic elements Cd, Pb, and essential metals Co, Zn, which are required for biological activities in cells however, it can be hazardous if their concentration (Mganga, 2014). Through redox activity, heavy metals can directly generate ROS, highly poisonous and without nutrient value, as well as indirectly induce oxidative stress by disrupting or degrading the organism's antioxidant defense mechanisms (Gutierrez et al., 2003; Valko et al., 2005).

Disturbances in heavy metal concentrations can cause cell damage, DNA destruction, and an imbalance in oxidative load. Furthermore, ROS targets macromolecules within the cell, such as DNA, protein, and lipids, causing aging and programmed cell death (apoptosis) (Valavanidis et al., 2006; Sohrabi et al., 2018).

The essential and non-essential elements can stimulate oxidative stress by generating ROS in living organisms. The intracellular ROS level is elevated after exposure to different concentrations of heavy metals (Lushchak, 2011). So they may affect the sterility or fertility of hydatid cysts, this approach was supported by current analysis statistically. The averages of concentrations of all the metals in the fertile and sterile hydatid cysts showed significant differences at P≤ 0.05. High levels of these metals in sterile hydatid cysts may impose an elevation in ROS levels in turn that led to an increase the oxidative stress which considers a driver of programmed cell death and infertility (Lushchak, 2011). This was confirmed by the study of interestingly Saadoon et al. (2014) found that the total level of antioxidants in the fertile hydatid fluid is higher than it is in sterile cysts, which supports the hypothesis of the role of oxidative stress in the sterility of hydatid cysts, and when the total level decreases of antioxidants are responsible for cell death in sterile cysts, as they do not block the effects of ROS.

There was no significant difference in heavy metals concentrations between sterile cysts isolated from the lungs and sterile cysts isolated from livers, P≥0.05, except Zn, (Table 4), which can be attributed to, Zn being an essential element required in cells' vital activities and has an important role in metabolic processes (Kamaruzzaman et al., 2010). In addition, this study confirmed that there was no significant difference between fertile cysts isolated from the lungs and fertile cysts isolated from the liver in all metals P≥0.05, (Table 5), suggesting that the level of heavy metals concentration may be responsible or one of the reasons for fertility, and sterility of hydatid cysts.

In another hand, heavy metals interfere with the biological activity of proteins such as heat shock proteins (HSPs) which act as molecular chaperones, through diverse mechanisms. In some cases, preventing refolding of the proteins (Sharma et al., 2011). This refolding prevention may be caused by the interaction between the rate of stress and protein response. (Narberhaus, 2002).

The presence of heat shock proteins was determined in sterile hydatid cyst fluids only, as well as the presence of high concentrations of the enzyme Cathepsin B, which is capable of activating the enzyme Caspases-3 (Vancompernelle et al., 1998; Paredes et al., 2007), that deemed as a key enzyme in the mechanism of apoptosis. It was detected in fertile and sterile cysts, but at a high level in sterile cysts (Paredes et al., 2007).

Present findings support the hypothesis that increased cellular stress and apoptosis cause the sterility of hydatid cysts due to increased accumulation of heavy metals (Paredes et al., 2007; Cabrera et al., 2008; Aziz et al., 2011; Pérez-Morales & Espinoza, 2015).

Conclusion

The biological parameters of hydatid cysts and their fertility, as well as the complete life cycle of *E. granulosus*, are all affected by environmental factors. Apoptosis, which is triggered by heavy metals through the production of reactive oxygen species (ROS), may be one of the main drivers of hydatid cyst sterility.

Conflict of Interest

The author hereby declares no conflict of interest

Consent for publication

The author declares that the work has consent for publication

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