Scolicidal Activity of Zirconium Oxide (ZrO_2) nanoparticles Against Protoscolices of Hydatid Cysts

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Abstract

Hydatidosis is a sickness that affects human and farm animals. This disease is deemed as a public health problem in different regions of the world until nowadays. Surgical overlaps is the best way to treat the disease, while the risk of surgery lies in the possibility of cyst rupture and leakage of protoscolices and the recurrence of infection again, this prompted researchers to use scolicidal agents before surgery such as ethanol, plant extracts, to reduce parasite spread and recurrence of infection, recently researchers have been using nanoparticles as a scolicidal agent, like gold nanoparticles, silver nanoparticles, selenium nanoparticles, and others. This research aims to evaluate the fatal effect of zirconium oxide (ZrO_2) nanoparticles to protoscolices of hydatid cysts. The Protoscolices were collected from sheep livers infected with hydatid cyst disease. The protoscolices were treated with different concentrations (250, 500, 1000, 2000, and 4000 µg/ml) of ZrO2 NPs. The viability of protoscolices was determined by using an eosin staining method after 15, 30, and 60 min. The results showed that the concentrations of 1000, 2000, and 4000 µg/ml were significantly effective in the killing of protoscolices after 60 min., where the fatality rate of protoscolices was 49.6%, 52.7%, and 53.1% respectively when compared with the control group 38.5% ($p<0.05$).

Key words: *ZrO_2, Zirconium oxide nanoparticles, protoscolices, Scolicidal, hydatidosis.*

Introduction

Echinococcosis is a zoonosis, including two types of hosts, definitive (carnivores) and intermediate (wide range of mammalian species). The parasites of genus *Echinococcus* are small cestodes (1-6 mm) [(1)]. The metacestodes of *E. granulosus* are cysts of different sizes filled with clear liquid and are called hydatids. The first discovery of hydatid disease dates back to the time of Hippocrates [(2)]. Cystic echinococcosis (CE) is a growing health problem in various parts of the world, including the Middle East [(3)]. The liver is the first organ in which the parasite settles and develops into the larval stage (hydatid cyst) [(4)]. The germinal inner layer of hydatid cyst represents the origin of protoscolices, which are the infective form to the definitive host, and the source of secondary infection when naturally or experimentally released within mammalian tissues or peritoneal cavity [(5, 6)]. Surgery and/or chemotherapy are a common treatment for CE, chemotherapeutic of this disease has been sophisticated in several animal model studies, and both albendazole and mebendazole are considered to have identical efficiency [(7)]. Surgery is used in special cases depending on the characteristics of cysts, such as large cysts that contain multiple daughter cysts, single superficial cysts at risk of rupture, and cysts interlaced with biliary tract. PAIR (Percutaneous, Aspiration, Injection and Re-aspiration) is an alternative way to surgery, with minimum risks [(8)]. Efforts were dedicated to finding out new protoscolicidal materials from plant sources, those efforts concentrated on some plant extracts that showed high effectiveness against CE [(7)]. Due to the unavailability of effective treatment for CE, there is an urgent need to find this treatment, so that the nanotechnology-based materials may be useful in the cure of diseases [(9)]. Nanotechnology is a technique that is concerned with the development and use of chemicals, devices, and systems of unfamiliar characteristics because of their small size (1-100 nm), in addition to their unique physicochemical properties which had great importance in many fields of research, including science and medicine [(10)].

ZrO2 NPs have been studied extensively because of their unique mechanical, thermal, optical, and electrical properties [(11)]. They have broad applications because

of remarkable biocompatibility, high strength, and low cost [12]. ZrO2 NPs are also able to possess noticeable antimicrobial properties [13].

Materials and Method

1- Collection of protoscolices.

Samples of hydatid cysts were collected from livers of naturally infected sheep in a slaughterhouse in Baghdad/Iraq. The hydatid fluid was withdrawn from the cysts by using a 20 ml syringe and transferred into flasks. The fluid was left for enough time to enable all protoscolices to precipitate. The germinal layers of the cysts were also collected and placed in a petri dish and washed very well to assemble all protoscolices. The protoscolices were suspended with normal saline. The viability of protoscolices calculated according to the method of of Smyth & Barrett [14].

2- Preparation of nanoparticles suspension.

Zirconium nanoparticles (29.8 nm) were purchased. Jeng & Swanson [15] method was adopted to prepare nanoparticles suspension with modulation, where 0.4g of nanoparticles were weighed and suspended in 100 ml of distilled water and sonicated for 20 minutes before use. Five concentrations of the ZrO_2 NPs (250, 500, 1000, 2000, 4000) µg/ml were prepared according to the method of Napooni et al. [16] with some modifications.

3- Solicidal activity of ZrO_2 nanoparticles.

The efficiency of five concentrations (250, 500, 1000, 2000, 4000) µg/ml of ZrO2 NPs were tested against protoscolices of hydatid cysts at different exposure times (15, 30, 60) min. 0.5 ml of protoscolices suspension (1000 ps) were mixed with 0.5 ml of each concentration of nanoparticles, then incubated at 37°C for 15, 30 and 60 min. Then the viability of Protoscolices calculated after exposure to each concentration/time.

4- Statistical analysis

The data were analyzed by using statistical software package IBM SPSS (version 25). The differences between experimental groups and control groups estimated by using ANOVA and T-tests at a significance level of 0.05.

Results

Fatality rate (%) of protoscolices after exposure to ZrO2 NPs at various concentrations following various exposure times as shown in Figure 1.

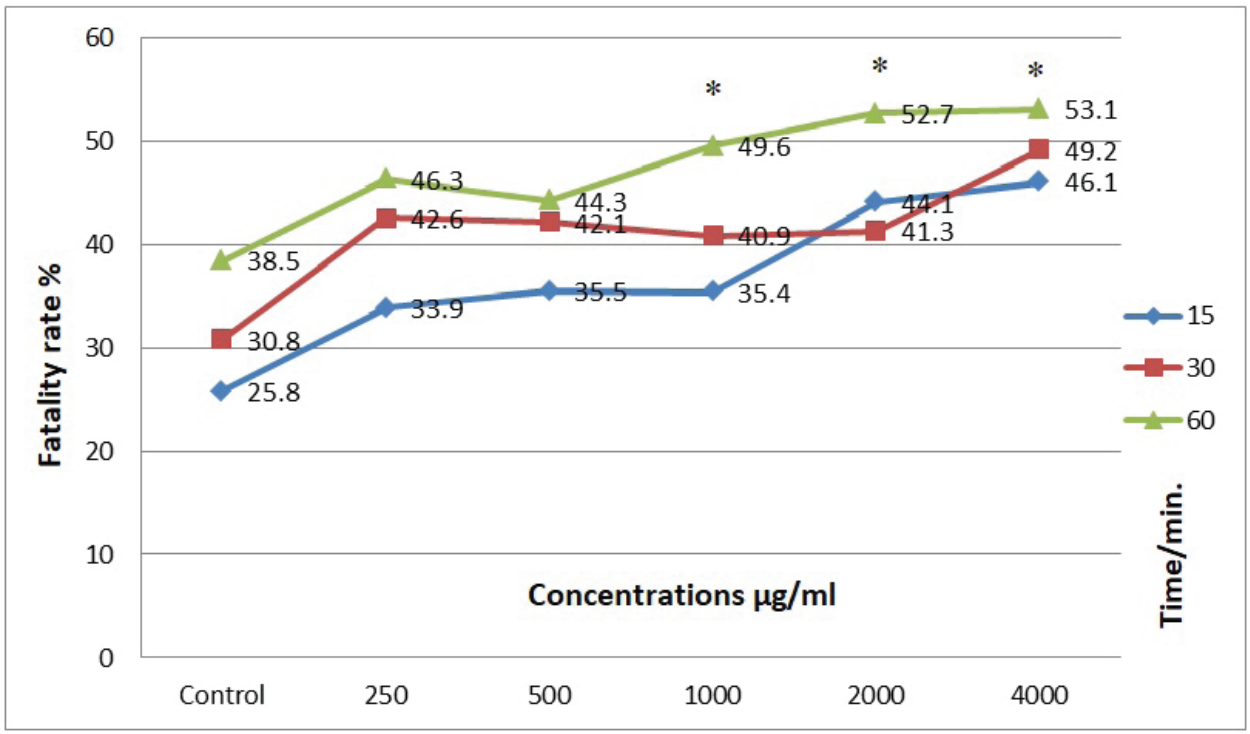

Figure 1: Fatality rate (%) of protoscolices after exposure to ZrO2 NPs at various concentrations following various exposure times.

* The difference between the experimental group and control group is statistical significance ($p<0.05$)

Discussion

The results of this study showed that the highest fatality rate of protoscolices was 49.6%, 52.7%, and 53.1% when treated with ZrO2 NPs suspension at concentrations 1000, 2000, 4000 µg/ml, respectively, after 60 min., and the difference between these groups and control group (38.5%) was statistically significant ($p<0.05$). Because of the importance of nanoparticles in medicine and science, the researchers studied its effects against some harmful organisms, especially bacteria and parasites, like the studies of Yalcinkaya et al. [17] and Kumaresan et al. [18], which proved the great effect of ZrO2 against *E. coli*, *Bacillus subtilis*, *Salmonella typhi*. Rahini et al. [19] studied the effect of green synthesis silver nanoparticles against protoscolices, and they found that the highest mortality rate was 90% (0.15 mg/ml, 120 min.). While the highest mortality rate was achieved when using the green synthesis gold nanoparticles (94%, 0.3 mg/ml, 120 min.) [20]. Naponi et al. [16] have also used gold nanoparticles at the same concentration for the current study and after 5, 10, 20, 30, and 60 min., they proved that all concentrations had a potential effect against protoscolices, while the highest mortality rate reached 76% (4000 µg/ml/ 60 min.).

Conclusion

It was concluded from this study that the ZrO2 was effective in the killing of the protoscolices of hydatid cysts, and the highest mortality rate reached 53%. It can be used other concentrations to elevate the efficiency of ZrO2 or combine these nanoparticles with plant extracts or drugs. It is suggested to study the effect of ZrO2 against hydatid disease in vivo, to ensure its safety and possible use as a treatment against this disease in the future.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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