Iraqi Journal of Veterinary Sciences

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Larvicidal effect of zirconium oxide nanoparticles in protoscoleces of hydatid cysts in the laboratory and mice

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Article information

Article history:
Received 06 August, 2023
Accepted 24 September, 2023
Published online 09 February, 2024

Keywords:
Hydatidosis
Nanomaterials
Metacestodes

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Abstract

The research focuses on the effect of zirconium oxide nanoparticles in the activity of protoscoleces of *Echinococcus granulosus* in the lab., seven strengths of the solution have been employed, 2.5, 5, 10, 15, 25, 50, and 100 µg/milliliter in distinct exposition durations, 10, 20, 30, 60, 90 minutes. Scottish mice have been inoculated with PSCs exposed to zirconium oxide nanoparticles of 5, 10, 50, and 100 ug/milliliter, for 30 minutes. Unimposing protoscoleces to ZrO_2 nanoparticles were administered to another group of animals as a controlling series. The whole experimental series has been anatomized after 3, 4, and 5 months after infestation. The results illustrated the apparent impact of zirconium oxide on the liveliness of protoscoleces, by increasing exposure time and strength concentricity in the laboratory, predominantly 29 and 0% at a concentration of 100 ug/ml at 60, and 90 minutes, distinctly. Likewise considerable diminished numbering of rising cysts in treated mice, mainly, at a concentration of 50 and 100 ug/ml in the fourth and fifth months 89.53 and 90.21% of invasion, with a presumed decrease in the diameters and weights of cysts in treated mice, exceptionally in the fifth month, in comparison with the untreated group. These observations presumed ZrO_2 nanoparticles, as a promising alternative agent against hydatid disease through future supplementary research.

DOI: 10.33899/ijvs.2023.142369.3175,

Introduction

Hydatidosis is an influential zoonosis parasitic malady induced by type tapeworms, metacestode of *Echinococcus granulosus* (1-3). It is considered a neglected disease even though it causes direct and indirect losses to (pets, wild animals), and humans (4). *E. granulosus* needs hosts as herbivores act as intermediate hosts to complete the parasite's life cycle and the infection is in the human acquired by the final host by swallowing eggs transmitted with the feces of the Canidae family, the final host (5). This disease causes economic losses in animal husbandry, and in humans causes significant consequences like surgery and other medical procedures. The disease spread worldwide, especially in areas with poor sanitation and living conditions, dealing with dogs and grazing sheep and other ruminants (6,7). Hydatid cysts mainly inhabit the liver and lungs. Ultrasound surveys in the United States have shown that cysts may grow from 1 to 5 mm per year or continue unchanged for years, they may rupture or disappear spontaneously (8). The medical operation remains superior to treatment, and other procedures perform dynamic action regarding the treatment of hydatidosis, not only for humans but also for dogs. Alternative strategies include treating the chemist as benzimidazoles especially for patients who do not endure surgery. However, this drug and other drugs showed severe adverse side effects, like bile inflammation and liver necrosis, and limited use due to low water solubility and the lack of gastrointestinal absorption make it inappropriate (9). Therefore, it was essential to proceed towards new ideal strategies against hydatidosis, Nanoparticles are efficient in lower concentrations and have the shortest exposure time, as

well as steady following reaction with metacestode liquid, not poisonous, extra functional, less hurtful to surrounding tissues (10). Nanomedicine is a comparatively novel domain of knowledge explored implementation in distinct areas like feed technologies, medication, transmission, remedy submission, and cosmetic operations (11-13). Nanometer size has a broad zone of medicinal objectives inclusively diagnosis and curative implementations in modernistic medication, visualizing, medicinal instruments, and inoculations (14,15). Nanoparticles have a small size ranging from 1 to 100 nanometers where NPs are helpful because of rising roof-size rate and individual physical together with chemical possessions, enabling the small size of nanoparticles to pass through a wide range of biological surfaces to reach target sites (16-19). Zirconium oxide nanoparticles are among the most promising and exciting metallic nanomaterials, whose properties differ significantly from existing ones. Zirconium is a transition metal element belonging to the titanium family. Nano zirconium dioxide has a high efficiency as an antimicrobial effect. It has anti-fungal, antioxidant and anti-cancer effects, with high chemical stability and corrosion resistance (20-24).

Most drugs, currently used for treating cystic echinococcosis have toxicity and side effects for humans or animals. The present study focused on employing ZrO_2 NPs as an alternative protoscolicidal agent in vitro and against hydatid cysts in experimental animals.

Materials and methods

Ethical approve

The study was accomplished in accordance with the declaration of Helsinki, and the protocol was approved by the Ethics committee of College of Veterinary Medicine at University of Mosul in ethical approval code UM.VET.2022.082 in 13/10/2022.

Decisiveness of hydatid cysts

Infested livers of lambs were collected from the butchery of Mosul city (Figure 1) after being detached at the laboratory, beneath disinfectant circumstances.

Collecting and assessing PSCs' liveliness

Protoscoleces of the larval cysts have been gained in the liver of stomachic lambs. Under aseptic conditions, the runny liquid and PSCs were secluded from the cyst bladders utilizing twenty-milliliter syringes while transmitted to vials. Liveliness extended from 98-100%. Protoscoleces were washed and centrifuged at 300 rpm for 15 minutes., three times with phosphate-buffered solution, pH 7.2, comprised, in the second wash, treatment together with antibiotics, ampicillin, and streptomycin. 20 µl of alive protoscoleces have been added to the exact extent of aqueous eosin 0.1% (25). Vitality has been evaluated beneath lighting microscope, and confirmed via bright greenish color and distinct movement of flame cells (Figure 2), while the dead protoscoleces absorbed the dye and appeared reddish (Figure 3).

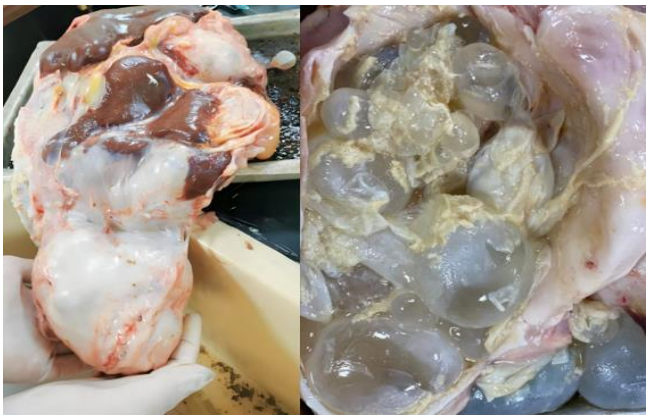

Figure 1: Metacestodees of *E. granulosus* in the liver of sheep.

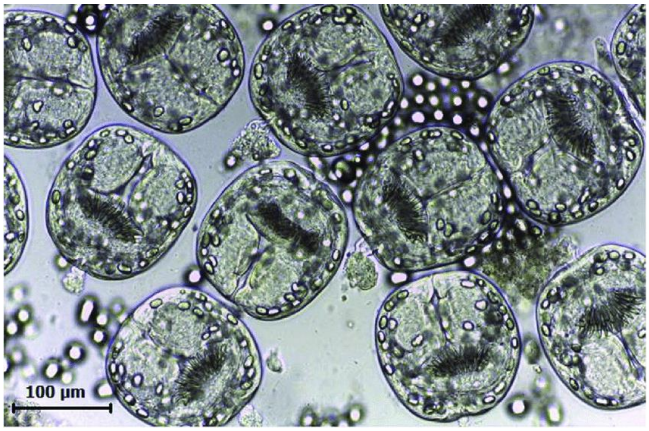

Figure 2: Lively protoscoleces.

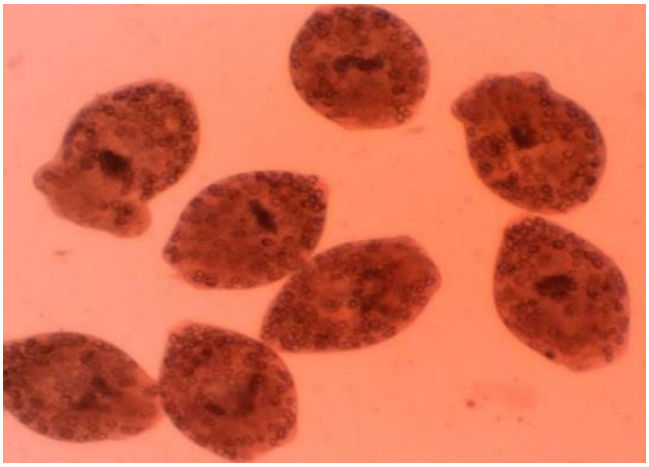

Figure 3: Dead protoscoleces.

Suspension of ZrO_2 nanoparticles

0.1 gram of zirconium nanoparticles, 50 nanometers, was hung with 100 milliliters of filtrated H_2O. After that sonication for 20 minutes (26).

Impact of zirconium oxide in the liveliness of protoscoleces in the laboratory

In order to investigate the effect of zirconium oxide versus s of the bladder cysts, 7 dilutions of ZrO_2, 2.5, 5, 10, 15, 25, 50, and 100 µg/milliliter have been used following variable exposition durations, by adding two thousand live protoscoleces (25) to the equal magnitude concerning every distinct dilution of ZrO_2 for 10,20, 30, 60, and 90 minutes, correspondingly. The admixture has been incubated at 37°C for the distinctly mentioned durations. Three replications were performed for each experiment to accomplish the most accurate counting concerning the viability of protoscoleces.

The action of zirconium oxide on bladder cysts in the mice

75 Scottish male mice, four to five weeks adult, were used and monitored in the animal house of the Faculty of Veterinary Medicine, University of Mosul. Along with unhandled series of protoscoleces 98 and 100% liveliness, handled protoscoleces with ZrO_2, 5, 10, 50, 100 µg/milliliter for 30 minutes. 60, 59, 52, and 40% have been chosen, and injected intraperitoneally into the mice. Five groups, five mice each, were designed. Two thousand of handled protoscoleces with ZrO_2 have been inoculated into the laboratory animals and the unhandled set, injected with 2000 vigor protoscoleces only. The whole series has been anatomized for three, four, and five months next to the invasion.

Statistics analysis

All data were accessed and resolved by complete random design to examine the influence of concentricity of ZrO_2 together with the duration of exposition, rather than the incorporation of them, by analysis of variance test. Extent divergence was estimated via Duncan multiple ranges test. Statistical analysis software, SAS, version nine was utilized (27).

Results

Effect of zirconium nanoparticles on protoscoleces *in vitro* and hydatid cysts *in vivo*

Table 1 elucidates the proportion of protoscoleces' liveliness in the laboratory, exposing them to distinct concentricity of zirconium oxide nanoparticles, for assorted durations. Table 2, utilizing DMRT, explains the considerable development of modest numbering of cysts in connection with untreated as opposed to treated series. Handling series revealed the lowest larvae numbering, 1.0 at 50 microgram/milliliter next to three- and five-months post infestation, and 100 microgram/ml next to four, five months as opposed to the control group, 6.4, 6.8, 7.2, consequently. Table 3 displayed marked varieties amidst median compatibility regarding exposure and duration. Larvae diameters diminished in handling set meaningfully, 0.05mm at 100 microgram/ml, as opposed to unexposed set, 2.619 millimeter five months next to infestation. Concerning weighing of bladder cysts, the whole handled series detected diminution meaningfully, 0.008 grams, in contrast with the unexposed set, 0.160 grams, five months after the invasion (Table 4). Table 5 illustrates the extremely low depreciation ratio of cysts numbering in handled mice, 90.21, 89.53, and 88.12%, at 100 microgram/ ml of ZrO_2, five months after the invasion (Figure 4).

Table 1: Distinction of protoscoleces liveliness proportion after exposure to zirconium oxide nanoparticles

Concentricity (µg/ml)	Exposing duration (minutes)				
	Ten	Twenty	Thirty	Sixty	Ninety
2.5	80%	72%	70%	51%	27%
5	77%	70%	60%	48%	22%
10	70%	68%	59%	43%	16%
15	72%	64%	53%	36%	0%
25	70%	60%	56%	38%	0%
50	65%	58%	52%	33%	0%
100	60%	45%	40%	29%	0%

Table 2: Effectiveness of zirconium oxide nanoparticles in numbering of cysts in handled, unhandled series

Duration	Three months	Four months	Five months	Mean
Unhandled	6.400a	6.800a	7.200a	6.800a
First group (5µg/ml)	2.000b	2.200b	2.800a	2.333b
Second group (10µg/ml)	1.400b	1.600b	2.600b	1.866b
Third group (50µg/ml)	1.000b	1.400a	1.000a	1.133b
Fourth group (100µg/ml)	1.200b	1.000b	1.000b	1.066b
Medium	2.400a	2.600b	2.920b	

different letters indicate significant differences, and similar letters indicate non-significant differences.

Table 3: impact of zirconium oxide nanoparticles on cysts diameter (millimeter) in handled, unhandled series

Time	3 months	4 months	5 months	Mean
Unhandled	1.4126c	1.7572b	2.6190a	1.9296a
1st series (5µg/ml)	0.1590gf	0.4840ed	0.6278d	0.4236b
2nd series (10µg/ml)	0.3046egf	0.1882egf	0.2870egf	0.2599b
3rd series (50µg/ml)	0.0950gf	0.3734edf	0.2620egf	0.2434b
4th series (100µg/ml)	0.0850gf	0.1840egf	0.0560g	0.1083b
Medium	0.4112a	0.5973a	0.7703a	

different letters indicate significant differences, and similar letters indicate non-significant differences.

Table 4: Effectiveness of zirconium oxide nanoparticle on cysts weighing (gram) in handled, unhandled series

Duration	Three months	Four months	Five months	Mean
Unhandled	.1600b	.2080a	.2120a	.1933a
1st series (5µg/ml)	.0300c	.0440c	.0300c	.0346b
2nd series (10µg/ml)	.0200c	.0260c	.0200c	.0220b
3rd series (50µg/ml)	.0080c	.0300c	.0100c	.0160d
4th series (100µg/ml)	.0120c	.0120c	.0080c	.010d
Medium	.0460a	.0640a	.0560a	

different letters indicate significant differences, and similar letters indicate non-significant differences.

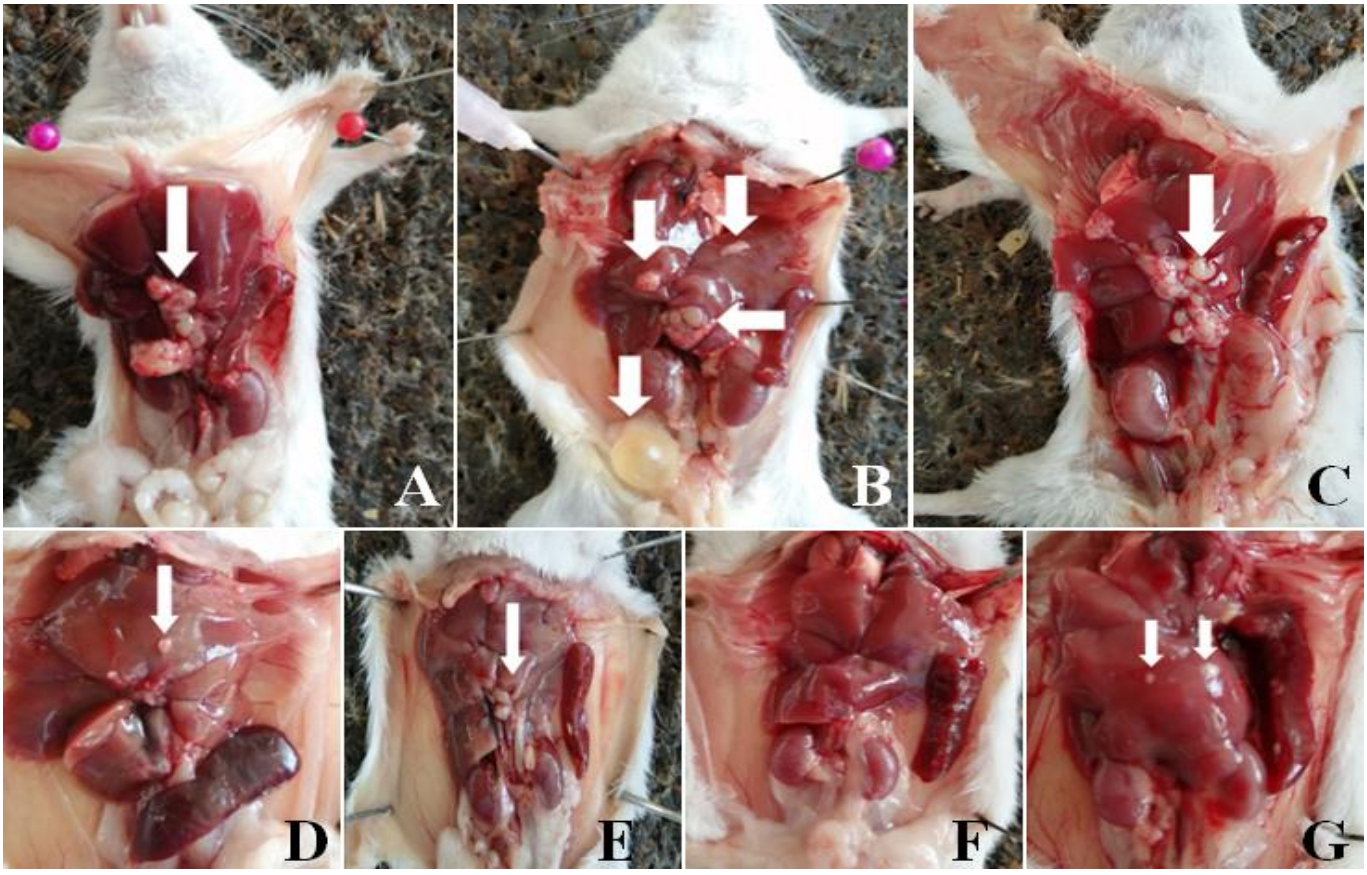

Figure 4: (A) Metacestodes of control series hereafter 3 months. (B) Metacestodes of unhandled series hereafter 4 months. (C) Metacestodes of control series, 5 months next to invasion. (D) Cysts in handled series at 50 µg/ml, 5 months next to invasion. (E) Metacestodes of handled series at 50 microgram/ml µg/ml, 3months next to invasion. (F) Deficiency of cyst bladder of series with 100 µg/ml, five months of infection. (G) Hydatid cyst processed mice with 100 µg/ml, 4 months postinfection.

Table 5: Proportion of lowering in the numbering of protoscoleces of handled series

Time	Three months	Four months	Five months
1st series (5µg/ml)	68.1%	83.5%	81.3%
2nd series (10µg/ml)	71.31%	85%	79.23%
3rd series (50µg/ml)	83.15%	87.21%	83.1%
4th series (100µg/ml)	88.12%	89.53%	90.21%

Discussion

Hydatidosis, as a parasitic sickness, is a prime difficulty involving pets, wild animals, and human worldwide, especially in areas with agriculture and animal husbandry (28). Recently, nanoparticles have been used increasingly individually and in combination with existing or conventional antimicrobial agents as an objective optional new remedy targeting cancer cells comprising detection, diagnosis, and therapy. Nanomaterials have unique properties that enable new therapeutic modalities beyond conventional drug delivery in the fight against cancer (29), parasites, and microorganisms that cause sickness (30,31). Medical operations represent the top option for remediation for sophisticated situations of cystic echinococcosis, but the spilling of protoscoleces leads to topical returning, larvae formation, and minor secondary dispersed echinococcosis (32).

Newly, many projects manifested nanoparticles have powerful mortal leverage in contrast to protoscoleces of *E. granulosus* (33-37). Others demonstrated the action of Zro_2 nanoparticles with malaria, leishmaniasis in disrupting the cell film within the parasite, producing H_2O_2 that leads to damage of cell membranes by releasing reactive oxygen species. Its therapeutic immunity lies in its cytotoxic effects, leading to immune responses that lead to programmed cell death (11,38,39).

The current project scouted decreased of numbers, periphery, and weights in cysts of animals vaccinated by protoscoleces treated with distinct zirconium oxide solutions for diverse intervals. The project outcome showed comparative divergence together with the study of Ibrahim (40) against protoscoleces of hydatid cysts, who reported zirconium nanoparticles lethality performance against the protoscolecs of *E. granulosus in vitro*, of 1000, 2000, and 4000 µg/ml, respectively, after 60 minutes, as the concentrations used were higher than the concentrations used in the current study. Investigators have proven the significant leverage of ZrO_2 nanoparticles contra gr+ve, gr-ve bacteria (41), nanoparticles do not immediately devastate nuclear film. Furthermore, they manipulate the cell's skeleton, alterations in inflammatory reactions, and exocytosis. Deterioration in cells and tissue could give rise to output reactive oxygen species.

Furthermore, heightened reactive oxygen species produced exterior interleukins, leading to extra deterioration. ZrO_2 nanoparticles produce an expansion yield of reactive oxygen species, multiplied caspase-3- three, mitochondrial film tension and deterioration, and deoxyribonucleic acid smash (42). Reactive oxygen species are manufactured via ordinary cell metabolisms, or every antigen, mitogen restraint, or as the output of breathing in mitochondria. In addition, while neutrocytes, phagocytic cells interact with opposite poisons and microscopic organisms, rather than interior stimulants (43). While reactive oxygen species scale overcomes on account of nanoparticles entrance, stimulation of immunity cells, rather than coding passages producing extra morbidity, like, inflammation, genome poisoning, excessive growth of fibrous tissues. NPs can overlap with cell coding passages out of 3 fundamental paths giving rise to programmed cell death operations (44,45). Many studies have shown the effects of nanoparticles of gold, silver, chitosan and oxidized metals on protoscoleces, the results indicate that the bioactive selenium nanoparticles in all concentrations have strong protoscolicidal effects, especially at 250-500 mg/ml after 10 and 20 minutes of application (46-48).

Conclusions

Mostly, the outcomes of this research elucidated that all concentrations of ZrO_2 nanoparticles' solutions, along with versatile durations have statistically significant divergences, as protoscolices substances in the laboratory, and empirical animals, taking into consideration the proportional rising of ZrO_2 concentricity and duration of exposition, on the protoscolicidal activity. These magnificent nanoparticles encouraged more research on implementing these substantial substances in surgical operations as substitutional complementary preferences.

Acknowledgments

The authors thank the Faculty of Veterinary Medicine, College of Education for the Pure Sciences, University of Mosul, for upholding and aperient.

Conflict of interests

The authors profess that they have no contradiction of interests.

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التأثير المميت لليرقات لأوكسيد الزركونيوم النانوي في الرؤيسات الأولية للأكياس العدرية في المختبر والفئران

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الخلاصة

تحرى البحث عن فاعلية أوكسيد الزركونيوم النانوي في عيوشية الرؤيسات الأولية للدودة المسطحة في المختبر، طبقت ٧ تراكيز مختلفة،٢,٥، ٥، ١٠، ١٥، ٢٥، ٥٠، و ١٠٠ ميكروغرام /مل ولفترات تعريض مختلفة: ١٠ ،٢٠ ،٣٠ ،٦٠ ،٩٠ دقيقة، على التوالي. حقنت الفئران السكوتلندية بالرؤيسات الأولية المعرضة لأوكسيد النانوي بالتراكيز ٥ ،١٠ ، ٥٠ ، ١٠٠ ميكروغرام /مل لمدة ٣٠ دقيقة. ، حقنت الرؤيسات الأولية غير المعرضة لأوكسيد الزركونيوم النانوي في مجموعة اخرى من الحيوانات التي اعتبرت كمجموعة سيطرة. شرحت جميع المجاميع التجريبية بعد ثلاثة، أربعة، خمسة أشهر بعد الاصابة. أوضحت النتائج تأثيرا واضحا لأوكسيد الزركونيوم النانوي في عيوشية الرؤيسات الأولية مع زيادة مدة التعريض وقوة التركيز في المختبر، بلغ أقصاها ٢٩ و ٠% عند التركيز ١٠٠ ميكروغرام/ مل بعد ٦٠،٩٠ دقيقة، على التوالي، إضافة الى انخفاض معنوي في أعداد الأكياس النامية في الفئران المعاملة، خاصة، عند التركيز ٥٠ و ١٠٠ ميكروغرام/مل في الشهر الرابع والخامس من الاصابة ٨٩,٥٣ و ٩٠,٢١%، ونقصان معنوي في أقطار وأوزان الأكياس في الفران المعاملة، وخصوصا في الشهر الخامس من الاصابة، بالمقارنة مع المجاميع غير المعاملة. تقترح هذه الملاحظات أن مادة الزركونيوم النانوية يمكن أن تطبق في المستقبل كعامل بديل واعد ضد الاصابة بداء الأكياس العدرية من خلال المزيد من الدراسات المستقبلية الإضافية.