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Effect of selenium nanoparticles against protoscoleces of *Echinococcus granulosus* in vitro and hydatid cysts in mice

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Abstract

This study determined the influence of selenium nanoparticles on the vitality of the protoscoleces of *Echinococcus granulosus in vitro*; seven concentrations were used: 50, 100, 150, 200, 250, 350, and 500 µg/ml for different exposure times: ten, twenty, thirty, and sixty minutes, respectively. Albino mice *Mus musculus* were injected with protoscoleces exposed to nanoparticles at 100, 150, and 200 µg/ml concentrations for 60 minutes. In contrast, control groups were injected with non-exposed protoscoleces. Mice were dissected three, four-, and five-months post infestation. Many criteria were relied on: numbers, weights, diameters of growing hydatid cysts, and their reduction proportion. The outcomes uncovered an apparent influence of selenium nanoparticles on the viability of protoscoleces of *Echinococcus granlosus* by the increase in exposure time *in vitro*, as well as diminish in the numbers of the larvae in processed mice versus the unprocessed collection; no cysts evolved inside processed mice at the concentration 200 µg/ml, three- and four-months post-infection. In contrast, the reduction rate was 90% in mice injected with exposed protoscoleces at the concentration of 150 µg/ml next 4 and 5 months of infection.

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Introduction

Echinococcus granlosus is a parasitic worm, that is considered as the central occasional agent for cystic echinococcosis which is caused by larval stage of the tape worm (1-3) that drives to abundant veracity issues rather than economical wastage in expanding nations because of the prices of diagnosis, medicine remediation and incompetence in several individual situations (4,5). Unilocular chinococcosis is one of prime reasons of economical wastage of domestic animal manufacture on account of morbidity and death rate of food generating animals (6). Hydatid cyst of the liver is usually asymptomatic, diagnosed accidentally or when there are complications such as infection or rupture (7). Life cycle of the tape worm comprises the terminal host, dogs, foxes, intermediate host - sheep, camel, pig, human, rodents as accidental host (8-10). The most effective method for treatment is surgical operation (11). hydatid cyst commonly infests liver 75%, other organs with different rates (12-15). Researchers explored that remediation with chemicals e.g., Albendazole, Mebendazole all correlated to lateral impacts (16). Nanotechnology, although not a new concept, has gained significant momentum in recent years. Nanomaterials are particles extent approximately between 1 - 100 nm. Technology of Nano is a protruding technique expected to allow novel opportunities versus microorganisms utilizing substances and apparatus at the atomic measure. Nanoparticles has received most attention as anti-parasitic drugs in few decades since the current anti parasitic drugs have side effects and their efficacy is not fully proved yet. Nevertheless, small attentiveness had paid to employ nanoparticles derivatives as antiparasitic drugs (17). Nanoscience research plays an important role in composition of efficient materials for combat diverse pathogenic microorganisms because of excess of antimicrobial resistance (18). Presently researchers explained that Nano

substances reveal exceptional features because of great ratio (surface -volume), furthermore nanoparticles can step inside the cell further extremely than another particle (19). Mineral nanomaterials are amongst major functional complexes in diverse medicinal areas like tumor remediation, aerobic illness remedy, neural remedy, contagious remedy, etc. (20). Those mineral substances possess protection properties versus bacteria, through generating Reactive Oxygen Species, albuminoid cohesion, film destabilized (21). Selenium (Se) is a major element of radical roles in human being veracity (22). yet, it has been utilized in distinct medicinal remediation, like tumor prohibition, comprising many body organs, further efficiency against viruses, and antioxidant impacts (23). Selenium possesses supplementary remarkable veracity activities efficiency concerning immunological restraint (24). Researches explored that Nanomaterials, essentially Selenium, could prohibit expanding distinct bacterial types (25). Many researches demonstrated that mineral Se interacts with film peroxidase for reproduction of distinct oxygenic free radicals like O^{-2} (26), moreover, the motivation capability of programed cell death of white blood corpuscles (27).

Therefore, recent study designed to reveal influence of Se against protoscoleces outside the body, further the prohibition of larval forms in experiential animals. This research is counted the first study in the country concerning implantation of selenium nanoparticles against hydatid disease in mice.

Materials and methods

Ethical approve OR data collection permit

All animal experiments of this research were accomplished under the approval of the ethical Committee of the Council of Education College for Pure Sciences in the first congress held on 1/10/2022.

Separation and experimentation of larval stages

The sheep infested liver gathered from slaughterhouse, in Mosul city (Figure1), then being separated in the laboratory under antiseptic conditions. Hydatid cysts were tested for viability.

Summation, estimation of protoscoleces viability

Protoscoleces (Pcs) of the tapeworm gathered infested organ, liver, were obtained under antiseptic conditions. Viability of (Pcs) was estimated according to Smyth and Barrett (28), 20µl of live protoscoleces were added to the same volume of 0.1% eosin, the viability was assessed under light microscope, then assured by the flame cells motion, impermeable action of exclusion aqueous eosin, colored bright green with characteristic motility (Figure 2), whereas the dead protscoleces absorbed the pigment, colored red (Figure 3). The viability ranged between 98 - 100%. The protoscoleces suspension was handled, through three washing processes with phosphate buffer saline (pH 7.2) comprising processing with antibiotics (Ampicillin and Streptomycin).

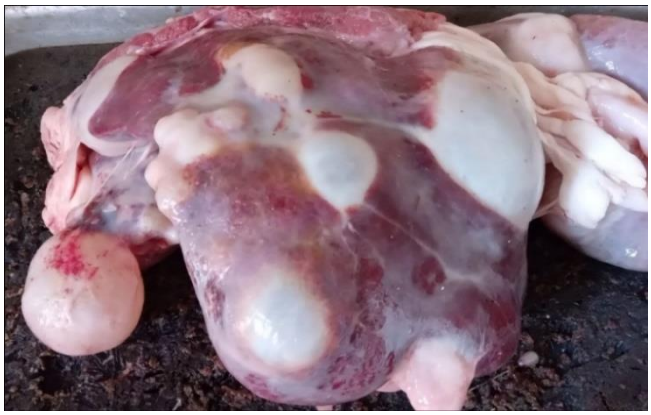

Figure 1: larval stages of *E. granulosus* in liver.

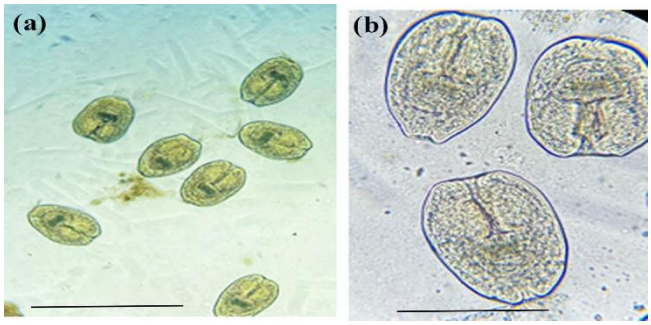

Figure 2: a, b: alive protoscoleces.

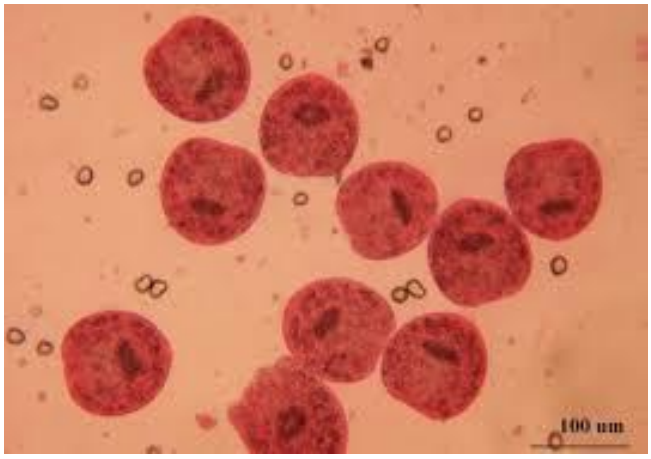

Figure 3: Dead protoscoleces. aqueous eosin 0.1%, 100X.

Experiential animals

Seventy-five male albino mice *Mus musculus* BALB/c, 4-5 weeks old, were being utilized and monitored, Collage of Veterinary Medicine, University of Mosul.

Effect of selenium nanoparticles on the viability of protoscoleces *in vitro*

To explore the impact of Selenium Nanoparticles against protoscoleces of larval stages, seven dilutions of Se, 50, 100, 150, 200, 250, 350, 500 µg/ml utilized together by variant exposure times, ten, twenty, thirty, sixty minutes. $2x10^3$/ml viable protoscoleces were added to the same volume of each distinct concentration of Se NPs for 10,20,30, and 60 minutes. The mixture was put in the incubator (37°C) for ten, twenty, thirty, sixty mins, respectively. Each experience was accomplished with three replicates (28). Viability was estimated under a light microscope.

Effect of selenium nanoparticles on hydatid cysts *in vivo*

Protoscoleces with viability 38.56%, 43.1%, 88.43%, and 90.83% were chosen for the experiential animals besides the control group with 98-100% viability. An overall of 75 mice, four to five weeks, were split into six collections (5 animals/set). The first set was inoculated with 2000 protoscoleces processed with the concentration 50 µg/ml of Se NPs for seconds, the second set was inoculated with 2000 protoscoleces processed with the concentration 100 µg/ml for 60 sec., the third set was inoculated with 2000 protoscoleces processed with the concentration 150 µg/ml for 60 sec, the fourth set was inoculated with 2000 protoscoleces processed with the concentration 200 µg/ml for 60 sec, the fifth one was inoculated with 2000 protoscoleces without processing (control), All collections were dissected three, four, five months post infestation.

Statistics analyses

Existing manuscript information has been analyzed due to CRD, for investigating dilutions rather than duration exposing influences, and the combination of the two factors. Range diversities has been evaluated by DMRT, SAS version 9 has been applied (29).

Results

Table 1 explains the mortality percentages of protoscoleces exposed to different concentrations of selenium nanoparticles, for different periods, in vitro. Table 2 detects alterations in the numeral, diameters and weightiness of the larvae, clarified F value, explored significant diversity concerning exposition and time.

Table 1: The variances for the mortality percentage of protoscoleces which exposed to selenium nanoparticles

Concentration (µg/ml)	Exposure time (min)			
	10	20	30	60
50	4.87%	15.17%	21.45%	38.56%
100	6.96%	20%	28.33%	43.1%
150	18.59%	28.8%	57.45%	88.43%
200	28.6%	52.5%	69.16%	90.83%
250	42.34%	68.5%	81.22%	94.5%
350	50.1%	85.16%	100%	100%
500	95.5%	100%	100%	100%

Table 2: impact of selenium nanoparticle on number, diameter, weight of larvae of experiential mice

Viability parameters		Time	Treatment	Time x Treatment	Standard error
	df	2	4	8	60
Cyst number	Sum of squares	0.96000	742.61333	4.90666	14.8000
	Mean squares	0.48000	185.65333 **	0.61333 *	0.2466667
	F value	1.95	725.65	2.49	
Cyst diameter	Sum of squares	95.0594586	404.651272	197.260208	4.8176800
	Mean squares	47.5297293 **	101.162818 **	24.6575260 **	0.0802947
	F value	591.94	1259.89	307.09	
Cyst weight	Sum of squares	0.08279289	0.18737841	0.12720874	0.76090122
	Mean squares	0.04139694 *	0.04684460 **	0.01590109	0.01268169
	F value	3.26	3.69	1.25	

* Average importance (P< 0.01) - ** average importance (P<0.05).

Table 3, by using Duncan's multiple range test, demonstrates diminution of moderate numeral of larvae concerning processed collection (Figures 5-9) in contrast to control animals (Figures 4 and 7). The processed mice displayed drooping in the number of cysts 0.000 after 60 minutes for the concentration 200 µg/ml, while control set revealed the highest cysts number 9.09, fifth month next infestation. Table 4 of rates exhibited remarkable diversity

concerning processed, unprocessed mice at 200, 150 µg/ml. Considerable diversity noticed between average compatibility exposition and period, cysts diameters of inoculated animals with protoscoleces processed by selenium nanoparticles diminished, 0.000 mm at 200 µg/ml exposure, 3th,4th month post infestation, followed by 150, 100 and 50 µg/ml respectively, contrast to the control set, 0.314,8.736 mm after 3,4 months. Concerning the weight of the hydatid cyst. Table 5 detected considerable diversity regarding moderates of compatibility related to periods and exposition. Weightiness of larvae diminished, 0.00 mg in 200 µg/ml, in processed animals (no cyst grows), followed by 150, 100, 50 µg/ml, respectively, versus control set (0.2856 mg) 4th month post infestation. Table 6 elucidated the elevated dropping rate of larvae numeral in processed animals at 200 µg/ml, then 150, 100 and 50 µg/ml, respectively.

Table 3: impact of selenium nanoparticles in the numeral of larvae of processed mice

Time	3 month	4 month	5 month	Median
Control group	8.60a	9.09a	29.98a	15.800a
First group (50 µg/ml)	2.20b	1.20cd	1.60bc	1.6667c
Second group (100 µg/ml)	1.40cd	1.2cd	1.20cd	1.2667c
Third group (150 µg/ml)	1.4cd	1.00cd	1.00cd	1.133c
Fourth group (200 µg/ml)	0.00e	0.00e	0.80d	0.2667d
Average	2.7200a	2.4800a	6.8800a	

The different letters indicate signification, identical letters show insignificance

Table 4: impact of selenium nanoparticles in larvae diameter(mm) of processed mice

Time	3 Month	4 Month	5 Month	Median
Control group	0.3140e	1.5020b	2.1760a	6.3840a
First group (50 µg/ml)	0.3360e	0.5560d	1.6200b	0.8373bc
Second group (100 µg/ml)	0.3080e	0.6800d	1.6740b	0.8873b
Third group (150 µg/ml)	0.3080e	0.6280e	1.0380d	0.6580c
Fourth group (200 µg/ml)	0.000f	0.000f	0.2880e	0.0960d
Average	0.25320c	0.67688b	1.35920a	

The diverse letters articulate signification at 1 % ($p< 0.01$), identical letters articulate insignificance.

Table 5: impact of selenium nanoparticles in larvae weightiness(gm) of processed mice

Periods	3 months	4 months	5 months	Median
Control group	0.17820ab	0.28560a	0.300bc	0.16487a
First group (50 µg/ml	0.08142bc	0.0740bc	0.0790bc	0.06094b
Second group (100 µg/ml)	0.05120bc	0.0772bc	0.0790bc	0.06913b
Third group (150 µg/ml)	0.0308bc	0.4162bc	0.13320bc	0.06827b
Fourth group (200 µg/ml)	0.00c	0.00c	0.03200bc	0.01067b
Average	0.25320c	2.120b	2.94440a	

The diverse letters articulate signification at 5% ($p<0.05$), identical letters articulate insignificance.

Table 6: percentage reduction of number protoscoleces in processed animals

Periods	3 months	4 months	5 months
First group 50 µg/ml	74.12%	86.98%	78.1%
Second group100 µg/ml	83.26%	90.96%	85.3%
Third group 150 µg/ml	83.4%	89.12%	88.62%
Fourth group 200 µg/ml	100%	100%	90.46%

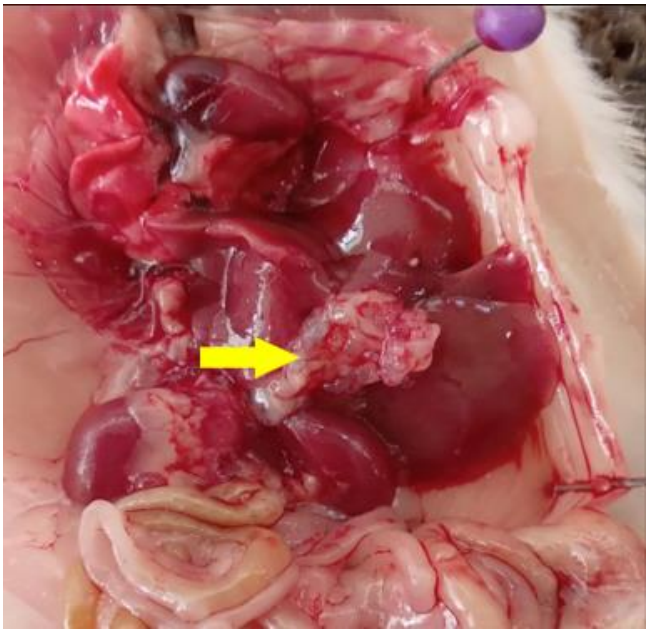

Figure 4: Hydatid cysts in control mice three months of infection.

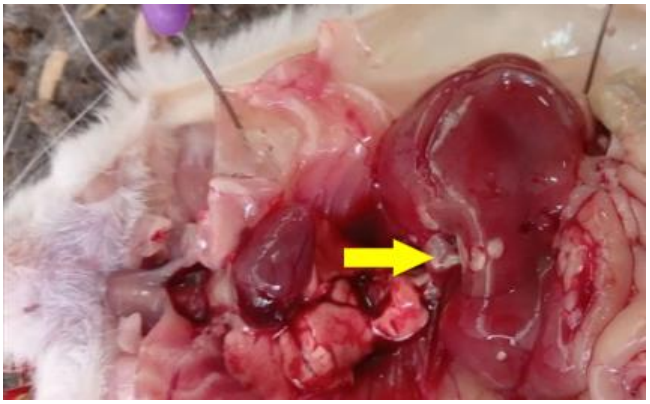

Figure 5: Hydatid cysts in first group mice with 50 µg/ml three months of infection.

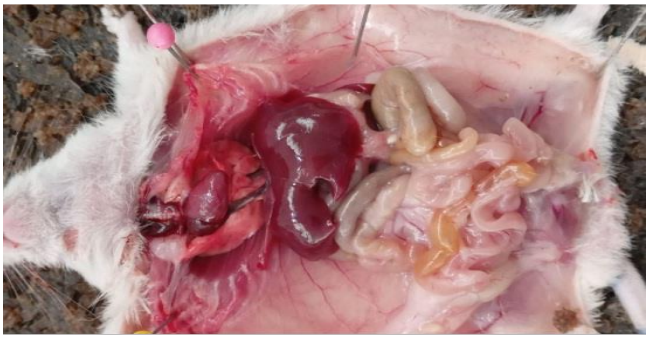

Figure 6: Lack of cysts in mice infested with protoscoleces at 200 µg/ml three months of infection.

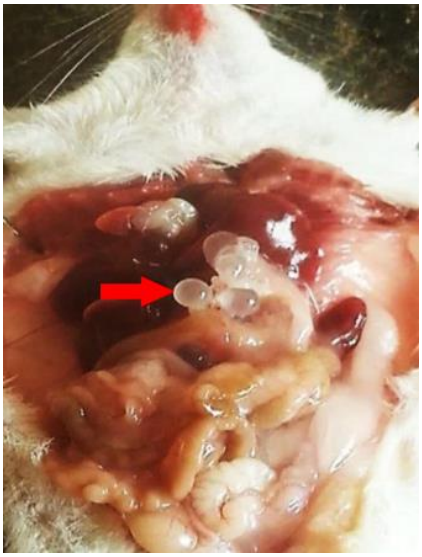

Figure 7: Hydatid cyst in unprocessed mice four months of infection.

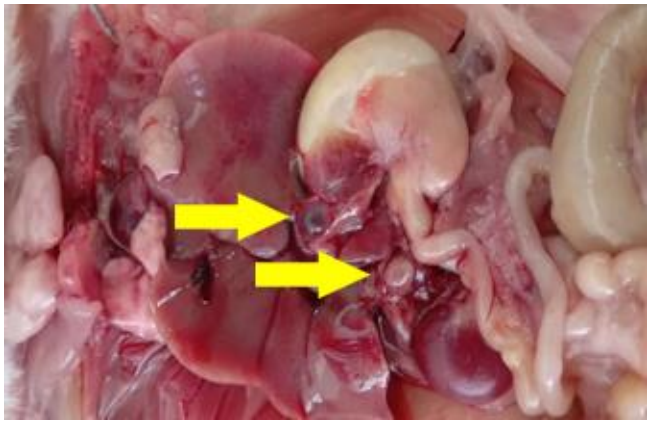

Figure 8: Hydatid cysts in processed mice with 50 µg/ml five months of infection.

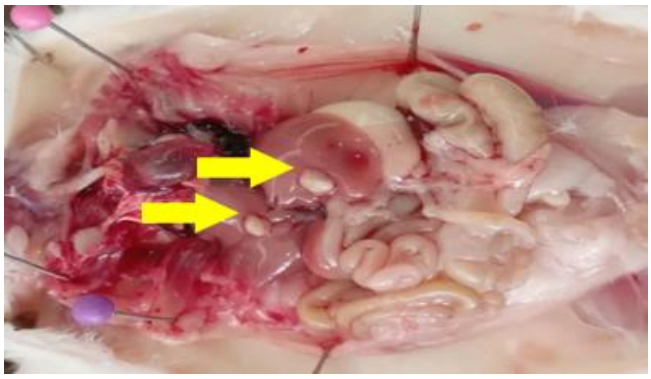

Figure 9: Hydatid cysts in processed mice with 100 µg/ml four months of infection.

Discussion

Taking into consideration lateral impacts of operative procedures concerning remediation of cystic echinococcosis, as formalization of neoteric larvae or passing because of effuse of larvae composition, insertion, aspiration, protoscoleces killing factors are proposed to exchange routines operative rote procedures. Numerous researches had been accomplished related to the killing impacts of many agents, as killers of protoscoleces inside the body, despite their lateral impacts as a remedy for unilocular hydatidosis (30).

Many techniques were used against infestation with hydatid disease in experiential animals, such as irradiation with laser, direct electrical current, probiotics and ultrasound (31-37), all techniques revealed considerable influence to the disease. All the techniques exhibited valuable efficiency against protoscoleces in vitro and cystic echinococcosis in mice.

Latterly, several researches exhibited Nanoparticles possess vigorous killing influence versus protoscoleces of hydatid cysts. Nanoparticles are a wide category comprising particulate materials, recalled growing medical attention, because of their capacity to transfer medication at typical dose scope, producing raised remediation effectiveness, minimized lateral impacts while improved patient acceptance (38).

Other studies have been done on Selenium (Se) nanoparticles, searching its different aspects because of reduction in acute (Se) toxicity, prompting of immune responses prompting programmed cell death of tumor cell, lower lateral impacts on natural cell, of programmed cell death in kidney diabetes individuals (39-41). Nanoparticle complexes possess killing influence versus bacteria, fungi, parasites, making those complexes a considerable portion in medication reproduction (42,43).

The results of the present study explored diminish in numeral, diameters, weightiness of larvae in mice inoculated with protoscoleces of E. granulosus that processed with various concentrations of Se NPs for various exposure times, throughout the experiments period, these results exhibited an agreement with other researcher's studies who applied nanomaterials versus parasites and other microorganisms in preceding researches that notified effectiveness of Selenium-versus cutaneous leishmaniasis outside and inside the body, displayed the nanomaterial prohibited reproduction of leptomonal and leishmanial forms of the parasite and restrict the disease in animals (26), furthermore, the same nanomaterial was utilized versus protoscoleces, demonstrated powerful proto scolicidal impacts, elucidating that the nanomaterial complex possessed powerful scolicidal impacts (27,44).

Ahmed Nematollahi (38) acted both Selenium and silver nanomaterials versus protoscoleces outside the body, the outcome of the study manifested differences between the two materials, elucidating that the killing capacity of silver nanomaterial was more than selenium. Other studies confirmed the pivotal influence of selenium versus trypanosomiasis, malaria (45,46). Many researches explored the prohibitory influence of different nanomaterials comprising golden nanomaterials against parasites (47,48).

Mechanisms for anticancer action, as chemopreventive and chemotherapeutic agent, are not fully understood, however, several hypotheses have been proposed: (i) enhanced oxidative stress, carcinogen detoxification, and immune surveillance; (ii) induction of cellular and mitochondria-mediated apoptosis; (iii) inhibition of tumor cell invasion and angiogenesis; (iv) cell cycle arrest at S phase; (v) metastasis prevention by inhibition of matrix metalloproteinases expression; and (vi) mobilization of endogenous copper (49,50).

Conclusion

In general, the feedback of the current study articulated that all concentrations of Selenium nanoparticles possess considerable protoscolicidal potency, consequently these nanoparticles are recommended as curative potent scolicidal agents in surgery against infestation with cystic echinococcosis.

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Conflict of Interest

No conflict of interest concerning publishing this research manuscript.

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تأثير مادة السيلينيوم النانوية على الرؤيسات الأولية للمشوكة الحبيبية خارج الجسم والأكياس العدرية في الفئران

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الخلاصة

حددت الدراسة الحالية تأثير مادة السلينيوم النانوية على حيوية الرؤيسات الأولية للمشوكة الحبيبية خارج الجسم، استخدمت سبعة تراكيز مختلفة: ٥٠، ١٠٠، ١٥٠، ٢٠٠، ٢٥٠، ٣٥٠، و٥٠٠ ميكروغرام / مل ولفترات تعريض مختلفة: ١٠، ٢٠، ٣٠، و٦٠ دقيقة، على التوالي. حقنت الفئران البيض بالرؤيسات الأولية المعرضة للمادة النانوية بالتراكيز ٥٠، ١٠٠، ١٥٠، و ٢٠٠ ميكروغرام/ مل لمدة ٦٠ دقيقة، في حقنت مجاميع السيطرة بالرؤيسات الأولية غير المعرضة، شرحت الفئران بعد ثلاثة، أربعة، وخمسة اشهر من الإصابة. اعتمدت معايير عدة منها التغيرات الحاصلة في أعداد وأوزان وأقطار الأكياس العدرية النامية والنسبة المئوية لاختزالها. بينت نتائج الدراسة أن تأثير مادة السيلينيوم النانوية على حيوية الرؤيسات الأولية ازداد بزيادة فترة التعريض خارج الجسم، فضلا عن اختزال أعداد الأكياس العدرية النامية في الفئران المعاملة مقارنة بمجاميع السيطرة، إذ لوحظ عدم نمو الأكياس عند التركيز ٢٠٠ ميكروغرام/ مل بعد ثلاثة وأربعة أشهر من الإصابة، بينما بلغت نسبة اختزال أعداد الأكياس ٩٠% في الفئران المحقونة بالرؤيسات الأولية المعرضة عند التركيز ١٥٠مايكروغرام/ مل بعد أربعة وخمسة أشهر من الإصابة.