

RESEARCH PAPER

Comparison of the Effect of Magnesium Oxide Nanoscale and Aqueous Extract of Neem Plant on the Primers of Echinococcus Granulomatosis

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ARTICLE INFO

Article History:

Received 11 January 2025

Accepted 23 March 2025

Published 01 January 2025

Keywords:

Nanoparticles

Magnesium oxide

Echinococcus Granulomatosis

AFM

ABSTRACT

Hydatidosis is a common disease between humans and animals and is caused by the larval stage of the tapeworm *echinococcus granulosus*, which is endemic in Iraq and neighboring countries. Therefore, the current study aimed to know the effect of magnesium oxide nanoparticles and neem plant extract on Protoscolex of *E. granulosus* isolated from sheep. Protoscolex isolated from the liver of sheep infected with aqueous sacs were incubated with extracts that were prepared in different concentrations at different intervals of 60, 30, 15, 10, 5 minutes. As for the aqueous extract of magnesium oxide, concentrations of 0.100, 0.050, 0.025 mg / ml were used, where the results showed a concentration of 0.100 mg / ml with a time of 60 minutes and treatment at a concentration of 0.050 mg / ml with a time of 60 Minute highest killing rate 95% and 98% respectively. And the aqueous extract of the dandelion plant has been used concentrations of 1000, 500, 250 mg / ml, where the treatment in sheep livers recorded concentration of 1000 mg / ml at the 60th minute the highest killing rate as it reached 93%, so significantly different from the rest of the treatments. By comparing the results of the effect of magnesium oxide nanotechnology with the plant extract, the results showed the superiority of magnesium oxide nanotechnology for all concentrations.

How to cite this article

Al-Douri R., Al-Douri M. Comparison of the Effect of Magnesium Oxide Nanoscale and Aqueous Extract of Neem Plant on the Primers of Echinococcus Granulomatosis. J Nanostruct, 2025; 15(2):400-406. DOI: 10.22052/JNS.2025.02.001

INTRODUCTION

Hydatidosis disease is one of the most serious health and epidemiological problems in most parts of the world, which Hippocrates first described as the bladder full of water [1]. Hydatid disease is a common human and animal disease Zoonosis, which is the larval stages of tapeworm belonging to the genus Echinococcus. [2].

Needs parasite *E. Granulosus* hosts to complete their life cycle as a final host, which is represented by carnivores from the canine family and a middle host, represented by herbivores [3,4]. The cause

of the disease in humans and intermediate hosts is the ingestion of food contaminated with tapeworm eggs [5]. The seriousness of hydatid cyst disease and its health importance lies in the fact that The symptoms of the disease do not appear in its initial stages, and there is no evidence of infection with the disease until after a period that may reach several years due to the slow growth of the cyst, as well as the increase in its numbers and large size, so the pathological symptoms of hydatid cysts appear due to the pressure of the cyst on the tissues adjacent toe [6]

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The seriousness of the disease, as well as the slow appearance of symptoms, lies in the treatment being limited to eradication For Hydatidosis with surgical intervention, which is the most effective treatment [7].

The importance of nanomaterial in its distinctive quantitative properties due to its small size and large surface. The ratio of the surface area of the nanomaterial to its mass is much greater than the same ratio in large molecules, which leads to a rapid chemical reaction [8].

Magnesium oxides are important oxides for scientific research and are used medically in the treatment of indigestion, the treatment of migraines, and the reduction of the risk of anxiety and stress, and it is used in industry to produce plates used in light engraving and in the installation of various battery walls, where little research focused on verifying the usefulness of particles compared to other nanoparticles [9].

MATERIALS AND METHODS

Physiological phosphate buffer solution (PBS)

It was prepared (Hamad, 2021) using the following substances: sodium chloride 8.00mg, aqueous disodium phosphate 2.89mg, potassium phosphate dihydrogen 0.2mg, potassium chloride 0.2 mg and distilled water (1) liter The above substances were gradually added in half a liter of distilled water and then completed the volume to 1 liter of distilled water, after which the pH (by adding a base) was adjusted to 2.7, and then sterilize the solution by placing it in the closed at a temperature of 121 ° C and under a pressure of 15 pounds / ang2 and for a third of an hour, keep this solution at a temperature of 4 ° C, used to save the heads.

Physiological brine

Prepared according to [10] using the following substances sodium chloride 9mg and distilled water (1) liter dissolve sodium chloride in half a liter of distilled water and then complete the volume to 1 liter, and sterilize using the closed at a temperature of 121 m for a third of an hour and under pressure of 15 pounds / ang2, save this solution at a temperature of 4 m used in washing the virgin sac.

Aqueous Eosin stain (1.0) %

The dye was prepared using the following substances: 0.01gm dye powder and 10ml distilled

water, the dye powder was dissolved in 10 ml of distilled water and kept at a temperature of 4 °C, the dye was used in measuring the vitality of primroses [3].

Formalin 10%

The solution was prepared by adding 10 ml of formaldehyde converter to 90 ml of distilled water [11].

Collection of hydatid cyst samples and isolation of heads

Hydatid cyst samples were collected from the liver of naturally infected sheep. The infected livers were transported to the animal laboratory in Mansourieh district / Diyala, by plastic bags in a container containing crushed ice form (1-3), and was dealt with directly following the method (1980) Smyth and Barrett. The affected organs were washed with water for the purpose of getting rid of blood and suspended materials from the slaughtering process and placing the liver in a sterile dish and the outer surface of the bag was sterilized with 70% ethyl alcohol and then the largest amount of hydatid fluid containing Protoscolex was withdrawn using a medical syringe of 3 ml. Using sterile scissors, the bag was pierced and the bag was washed with a phosphate brackish buffer solution to remove the rest of Protoscolex deposited or stuck in the bag. After collecting the heads, they were placed in test tubes for the purpose of deposition.

Determination of vitality and number of primates

The vitality of the heads was estimated and their numbers were calculated at the same time by taking 10 µL of the capric suspension with a similar volume of aqueous eosin dye at a concentration of 0.1% using a fine pipette and after shaking the solution a drop was taken from it and examined under a light microscope and the percentage of living heads that appeared in bright green was calculated due to the process of eosin exclusion compared to the dead primary heads that appeared in red and With an average of three readings [12].

Chemical used

Dissolve 0.9 g of magnesium chloride aqueous salt $MgCl_2(H_2O)_x$ in 50 ml of non-ionic water and put the solution on a magnetic motor for 30 minutes until the substance is completely

dissolved, prepare a solution of sodium hydroxide NaOH by dissolving 1.6 g of it in a quantity of distilled water and then complete the volume to 200 milliliters of non-ionic water, add 150 milliliters of this solution to the cobalt solution with continuous stirring using the engine and at a temperature of 80 ° C with monitoring not to rise pH more than 9 Throughout the reaction period, dry the precipitate for 30 minutes at 80°C and then burn at 400°C for 2 hours and leave in the dryer for 24 hours at room temperature, and the appropriate solution was obtained Stock Solution at a concentration of 0.1 mg/ml, from which the rest of the concentrations (0.025, 0.050) mg/ml were prepared using the law of $V_2 C_2 = V_1 C_1$ [13].

Source of plant used in the study

The neem that was used in the preparation of the aqueous extract was obtained from the fields of Diyala Governorate Mansouria District. The aqueous extract was prepared according to the method [14].

Take 30 g of plant leaves for each of the plants tested under study and after washing placed in an electric mixer type National and add to it 250 ml distilled water and then mixed for 15 minutes and leave the mixture half an hour filtered the mixture by a boring cloth to separate plankton, then

transferred to the centrifuge (Centerfuge) type Hettich after being placed in test tubes and at a speed of 3000 cycles / minute for 15 One minute. Filter the extracts in sealed bottles and kept in the refrigerator at 4°C until use.

Synthetic test results

X-ray diffraction results

For the purpose of ascertaining the size of the finished nanomaterial and to ensure that it is completely dissolved, X-ray diffraction technique was used to determine the crystal shape of the nanoparticles and their composition, as well as to calculate the size of the nanoparticles according to the Debye-Scherre equation [15]:

$$D = \frac{k\lambda}{\beta \cos \theta} \tag{1}$$

X-ray diffraction of magnesium oxide nanoscale

The results of the X-ray analysis in the magnesium oxide test shown in Fig. 1 confirmed their conformity with the international card. We observe different peaks at $2D = 34.4540, 31.8005, 36.28822$ From the figure, it is clear and based on the results of X-ray diffraction that magnesium oxide nanoparticles are pure, because the overlapping peaks of impurities do not appear and

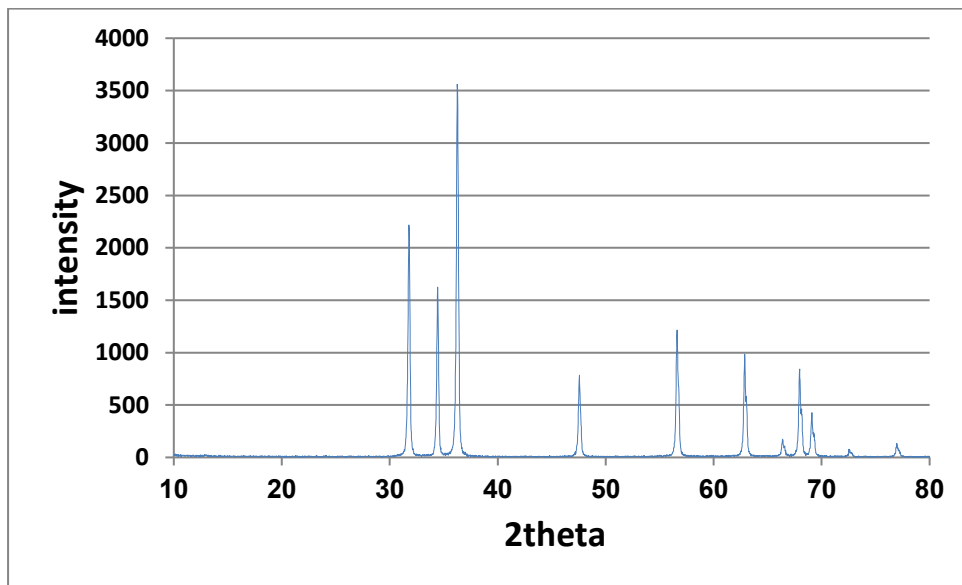


Fig. 1. X-ray diffraction model of magnesium oxide nanoscale powder

that the average size of the nanoparticles used in the study is equal to 51 nm, and these results are consistent with the study of [16] Those who showed that the peaks of the diffraction angle were equal in their study to 56.60, 47.53, 36.25, 34.42, 31.76, and this corresponds well to the Joint Committee on powder Diffraction Standards (JCPDS), which indicates the presence of the same peaks and thus indicates the quality of the nanomaterial obtained from the market.

Atomic Force Microscope (AFM)

We note through Fig. 2 that the measurements of atomic force microscope (AFM) for MgO molecules, as the highest value of plate thickness is 697 nm and is three-dimensional and the length of the plate increased by 6.19 μm , the number of minutes examined was 136 and the average size of the minutes was 71nm, and from the above measurements, it was confirmed that magnesium oxide was nano-sized.

RESULTS AND DISCUSSION

Effect of Magnesium Oxide Nanoparticles on the Biology of Primary Heads of Granular Echinococcus

The results in Table 1 show the significant effect on the vitality of Protoscolex of the granulosus echinococcus, as the average concentration showed that there is a significant difference between the concentrations used to kill the vitality of the heads and the concentration of 0.100 mg/ml achieved the highest killing rate, which reached 62.80%, which differed significantly from the concentrations of 0.050 and 0.025 mg/ml, which recorded a killing rate of 54.60% and 48.80% respectively.

From the same table, we find that there is a significant difference between the average time in killing the vitality of the heads, as the time recorded 60 minutes the highest rate of killing, which amounted to 94.00 Superior morally than the rest of the times.

As for the effect of interaction between the two factors, the treatment at a concentration of 0.100 mg/ml with a time of 60 minutes and a treatment with a concentration of 0.050 mg/ml with a time of 60 minutes showed the highest killing rate of 95% and 98% respectively, which differed significantly from the rest of the treatments. While the treatment at a concentration of 0.025 mg/ml with a time of 5 minutes recorded the lowest

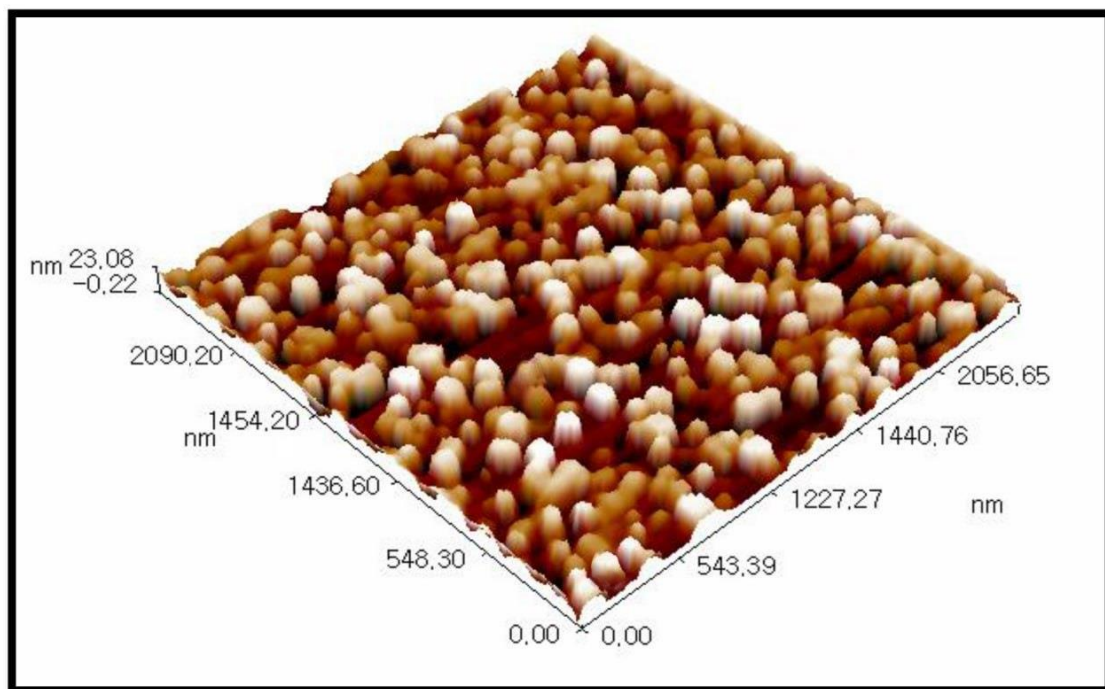


Fig. 2. Three-dimensional image of magnesium oxide nanoparticles by atomic force microscopy

killing rate, which amounted to 10%.

We find from the table that the best concentration of cobalt oxide nanooxide in killing

Protoscolex Echinococcus granulosus is 0.100 mg / ml at 60 minutes and therefore the relationship is direct with time and the reason for killing the

Table 1. Effect of Cobalt Oxide Nanoconcentrations on the Biology of Primary Heads of Granular Echinococcus

Average time	Concentration 0.1	Concentration 0.05	Concentration 0.025	Time concentrations
13.66 E	20 h	11 l	10 l	5 minutes
32.00 D	35 f	33 fg	28 G	10 minutes
56.33 C	68 d	53 e	48 E	15 minutes
79.33 B	88 b	81 C	69 D	30 minutes
94.00 A	98 a	95 a	89 B	60 minutes
	62.80 a	54.60 b	48.80 C	Average concentration

Lowercase letters that are similar horizontally mean that there are no significant differences between them. Vertically similar capital letters mean that there are no significant differences between them.

Table 2. Effect of Aqueous Extract Concentrations of Dandelion Plant on the Vitality of Primary Heads of Echinococcus Granulomatosis

Average time	Concentration of 1000 mg	concentration 500 mg	Concentration 250 mg	Focus Time
10.33 E	13 Hi	10 Hi	8 I	5 minutes
24.66 D	34 G	26 H	14 H	10 minutes
41.33 C	63 E	31 G	30 G	15 minutes
64.33 B	83 b	62 E	48 F	30 minutes
82.66 A	93 a	82 C	73 D	60 minutes
	57.20 A	42.20 B	34.60 C	Average concentrator

Lowercase letters that are similar horizontally mean that there are no significant differences between them. Vertically similar capital letters mean that there are no significant differences between them.

heads with magnesium oxide nanoparticles may be attributed to the decrease in surface agglomeration and the provision of more surface area to interfere with parasitic membranes, which leads to more toxicity.

The results of the treatment of primers outside the vivo with magnesium oxide nano extract were similar to the results obtained by (2024) Mahmoud Effect of Zirconium Oxide on the Biology of the First Heads of Granulomatosis Where seven different concentrations were used (2.5, 5, 10, 15, 25, 50, 100) $\mu\text{g/ml}$ and exposure times (10, 20, 30, 60, 90) and the concentration of 100 $\mu\text{g/ml}$ achieved the highest killing rate of 90%. The results were similar to (2023) Mahmoud Effect of cobalt oxide nanoparticles on the biology of the initial heads of echinococcus, which was used by different coding and different times, where the concentration of 0.100 mg/ml with a time of 60 minutes achieved the highest killing rate of 98%. It was also similar to the study of [17-20] the effect of selenium nanoparticles on the vitality of primers of echinococcus granular particles in the laboratory, seven concentrations of 50, 100, 150, 200, 250, 350, 500 $\mu\text{g/ml}$ were used for different exposure times 10, 20, 30 and 60 respectively and the concentration of 350, 500 with time 60 was obtained 100% kill. The results of the current study are consistent with those obtained by Shnawa et al. [21] In the activity of zinc oxide nanoparticles biosynthesized from mint leaves against Protoscolex of the granulomatosis, which obtained a 100% killing rate at a concentration of 400 ppm for the time 150 min. The results of the study converged with the study of Abud Al Aziz et al. [22] The development of gold nanoparticles as a drug against cutaneous leishmaniasis in vitro The study showed effectiveness in inhibiting parasite growth, as it showed particles with a size of 0.15 μL , with an average growth of 1.56×10^6 cells/two files, with a time of 96, while the size showed 0.20 The highest inhibition of parasite growth with an average growth of 0.85×10^6 cells/ml at a time of 72 from the beginning of the experiment.

Effect of Neem Plant Aqueous Extract Concentrate on Primers of Granulomatous Echinococcus

The results of the statistical analysis of the average concentration in Table 2 on the effect of aqueous extract of neem plant on the vitality of primates show that the concentration of 1000 mg / ml differed significantly from the rest of the

concentrations with a killing rate of 57.20%. As for the effect of average time on the vitality of the heads, the time of 60 minutes achieved the highest average killing rate of 82.66%, superior to the rest of the times. As for the effect of worker interaction, the treatment at a concentration of 1000 mg/ml with a time of 60 minutes showed the highest killing rate of 93%, which differed significantly from all treatments, while the treatment at a concentration of 250 mg/ml with a time of 5 minutes recorded the lowest killing rate of 8%.

From the table, we find the best concentration of killing Protoscolex of aqueous grape seed extract is 1000 mg/ml at minute 60, with a killing rate of 93%. The effect may be attributed to dandelion leaf extract, which is a rich source of a variety of active substances and compounds including beta-carotene, carotenoids, xanthophyll, chlorophyll and vitamin C, D, B A, E, Choline, minerals such as iron, silicon, magnesium, sodium, potassium, zinc, manganese, copper, phosphorus, the presence of natural compounds of phenols, flavoins, inulin, lactones and glycosides, and the high content of minerals, fiber, vitamins and essential fatty acids make it a preferred food source [23], which may affect the vitality of primroses by interfering with metabolic activities. Inside the cell with enzymes, proteins, nucleic acids, carbohydrates and others. The current study is consistent with several studies that have shown the role of dandelion aqueous extract and its role as an antiparasitic. Where the results of the study coincided with the results of the study of Mares et al. [19] in evaluating the effect of the oleander leaf extract on the vitality of Protoscolex of the granulomatosis, where concentrations of (10, 30, 50) mg / ml of the extract and exposure times (5, 10, 20, 30) minutes were used, and the concentration of 50 mg / ml with the time of 30 minutes obtained the highest killing rate of 92.3%. The results of the study converged with the results obtained by [24] his study on the effect of the aqueous extract of the guava plant Psidium guajava on the vitality of primers in vitro and its use in different concentrations mg/ml 100, 75, 50 mg/ml at different incubation times 48-6 hours, the plant aqueous extract recorded a 100% killing rate at a concentration of 100 mg/ml with an exposure period of 48 hours. The results were similar to Yadegari et al. [25] in a study on the effect of Silybum marianum on the vitality of Echinococcus granulomatosis, and the

concentration of 500 mg over time 60 proved the highest killing rate of 77%.

CONCLUSION

In conclusion, the study demonstrates that both magnesium oxide nanoparticles and neem plant aqueous extract significantly impact the vitality of Protoscolex of *Echinococcus granulosus*, with optimal concentrations and exposure times yielding high killing rates. These findings underscore the potential of these substances as effective antiparasitic agents, warranting further exploration for their application in controlling echinococcosis.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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