International Journal of Forensic Medicine

E-ISSN: 2707-4455
P-ISSN: 2707-4447
IJFM 2022; 4(1): 08-12
Received: 06-11-2021
Accepted: 09-12-2021

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Effect of garlic extract loaded on zinc oxide nanoparticles against *Sarcocystis in vitro*

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DOI: https://doi.org/10.33545/27074447.2022.v4.i1a.41

Abstract
The present study aimed to investigate the effect of garlic extract loaded on zinc oxide nanoparticles on the viability of *Sarcocystis in vitro* Samples of the parasite in the study was divided into three group inside to control group each one consists of 20 parasite in petri dish (5 petri dish) for each concentration contain the Phosphate Buffer Saline(PBS). The group treated with one concentration of the hybrid nanoparticles (Garlic extract loaded on zinc oxide nanoparticles) (0.1, 0.3, 0.5) mg/ml in 4 ^{o}C and noticed the viability for five days. The nanoparticle measuring by SEM (Scaning Electronic Microscop) in Karbala University, College of Veterinary Medicine, the results showed that process of loading garlic Extract on the surface of zinc oxide nanoparticles was succeed and the hybrid nanoparticles had great effect (100%) in mortality of the parasite in high concentration 0.5 mg/ml followed by 0.3 mg/ml (8.98%) and (17.66%) in 0.1mg/ml. The statistical analytic results showed that there was significant increasing in mortality in all concentrations.

Keywords: Nanoparticles, zinc oxide, *Sarcocystis*, garlic extract

Introduction
The relationship between man and plants extends to the beginning of the emergence of the human race, and that evolution and the complex life that accompanied it opened many horizons in the fields of using plants, which increased their importance generation after generation. The civilization of Mesopotamia, the civilization of the Nile Valley and the ancient Indians practiced the profession of herbal medicine and perfected it, as well as the Greeks who put the literature on herbal medicine during the fifth and fourth centuries BC (James *et al*., 2012) [47]. Plant that has historically been used for the treatment and prevention of some diseases, Garlic has many pharmacological properties, such antimicrobial antioxidant and anticancer activity. Garlic and its components have potent anti – parasitic activities against many human and animal parasites such as Leishmania (Wabwoba *et al*., 2010) [48]. Schistosoma Trypanosoma, Giardia, Entamoeba and Plasmodium Furthermore, garlic has immunostimulatory activities, Also, garlic is able to act as anti – oxidant for inhibiting lipid peroxidation leading to hepatoprotection (Hijran *et al*., 2019) [22].
Sarcocystis parasite is one of the primary animals that cause a kind of common diseases between humans and animals, Zoonotic diseases that spread widely in the world so-called Sarcocystosis, It produces more than 200 known species of myocystosis depending on the type of infection and the nature of the material ingested (Well cooked or not purified) by the human. *Sarcocystis* is one of the widespread protozoa in the world, as it forms cysts of different sizes and shapes within the tissues of the intermediate hosts that include humans and a large number of animals such as mammals, birds and reptile. Sarcocysts are obligatory parasites, as they need hosts to complete the life cycle. Herbivorous, plant and omnivorous carnivores are intermediate hosts for the disease, while carnivorous carnivores are definitive hosts of the parasite (Michael, 2017) [26]. The nanotechnology is deals with materials with nanoscale sizes, which are called nanoparticles, and they are materials that have crystals with sizes of one billionth of a meter, The strength of nanotechnology today lies in the widespread application of nanoparticles and that the symbol NP is used to describe one or more components that have at least one dimension in the range of 1-100 nanometers (Tjong & Chen, 2004) [49]. Great surface properties, and the surface of any of these materials has many reactions, in which the surface activity is very high due to the large surface area to volume ratio of NP, and it is possible that these materials are used in quantities much less than the

usual case and the amount of surface area is large, allowing them to react Fast actions in less time, so many properties of different materials can be changed at the nanoscale, and here lies the strength of nanotechnology (Williams, 2008) [44]. And that the toxicity of NP to cells and the response of other cells depends on the nature of these substances and the doses used, and that liver cells have a significant effect on the metabolism of these compounds and their removal from the blood easily.

Aim of the study

The present study was to investigate the effects of garlic extract loaded on zinc oxide nanoparticles in viability of *Sarcocystis in vitro.*

Materials and methods

Collecting samples for the experiment

Parasite sample collected

Samples from the esophagus muscles of sheep Infected with *Sarcocystis* parasite were collected from the Karbala butchery after confirming that they were infected with the parasite through macroscopic and microscopic examination. They were transported in refrigerated containers to the laboratory.

1. Preparation physiological solution

1. Phosphate Buffer Saline (PBS)
2. Preparation of this solution using the following materials:
3. Sodium chloride (8g)
4. Sodium phosphate monohydric (2.89g)
5. Potassium chloride (0.2g)
6. Potassium phosphate dihydrogen (0.2)

The above materials are gradually dissolved in 10 ml of distilled water and placed in piker of 1000 ml capacity and complete the volume to 1 liter of distilled water at a pH of 7.2 and sterilized with an autoclave at a temperature of 121 °C and pressure atmosphere for 30 minutes. And stored in the refrigerator at a temperature of 4 °C (Hudson & Hay, 1989) [23].

2. Preparation of water garlic extract

Fresh garlic (90 g) were mixed with demineralized water (1000 ml) and grinded in a blender for 15 min. the solid parts of the garlic were removed by filtration through sterile gauze, followed filtration bt filter paper followed by centrifugation at 4500 rpm for 30 min at 20 c the supernatant was filtrated through filter paper then the extract was dries in oven for 15 min at 80 c. finally, the extract was collected and stored in dry place.

3. Preparation of (Zinc oxide) Nanoparticles

Preparation of zinc oxide nanoparticles by using Poly Alcohol (PVA) as surfactant to prepare zinc oxide nanoparticles 0.01% PVA solution initially prepared and 2ml PVA was added to 1M zinc sulphate heptahydrate solution and 2M sodium hydroxide was added to it drop wise very slowly the resulting solution is stirred for almost 18 hours.

After 18 hours white precipitate was formed which was filtered and washed with distilled water and dried in a muffle furnace at a temperature of 100 °C for 2 hours then it was ground to fine powders and finally calcite at 450 °C. The sample of nanoparticles was examined by SEM (Scaning Electronic Microscop) in Karbala University, College of Veterinary Medicine.

4. Studying the effect of the hybrid compound (Garlic extract loaded with zinc oxide nanoparticles) on the viability of the *Sarcocystis* parasite *in vitro*.

The effect of the hybrid nanoparticles was studied *invitro*, as the physiological solution (phosphate Buffer Saline) containing 20 parasite was distributed with a volume of 10 ml in each Petri dish,5 petri dish for each concentrations, then these dishes were treated with concentrations of(0.5, 0.3, 0.1) mg/ml of the hybrid compound (garlic extract loaded on zinc oxide). The parasite's vitality was calculated for five days in temperature 4 °C and the percentage of Mortality Rate was calculated.

Statistical analysis

The results were statistically analyzed using ANOVA with the least significant difference (L.S.D) at a significant level ($p< 0.05$) (2000,الراوي وخلف الله).

Result &Discussion

1. Preparation of Nanoparticles

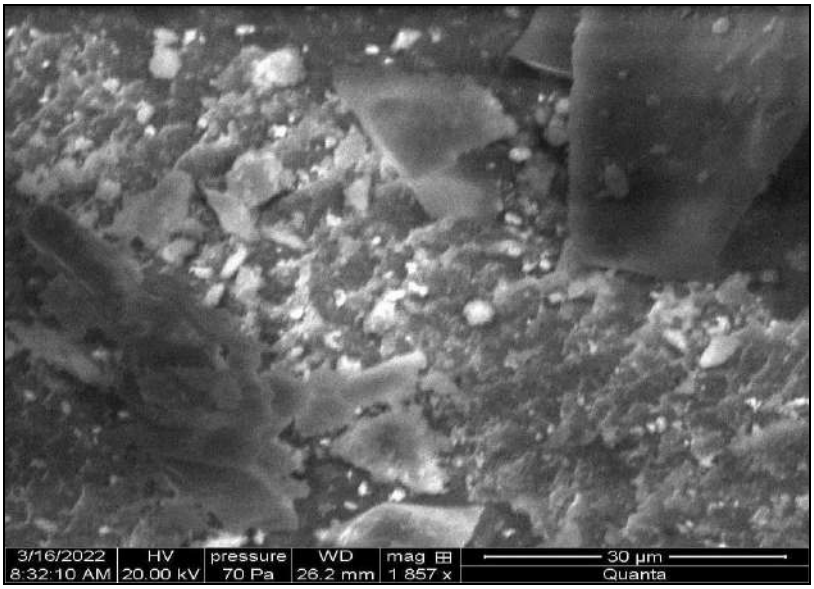

Fig 2: SEM image of free zinc oxide prepared using PVA as surfactant

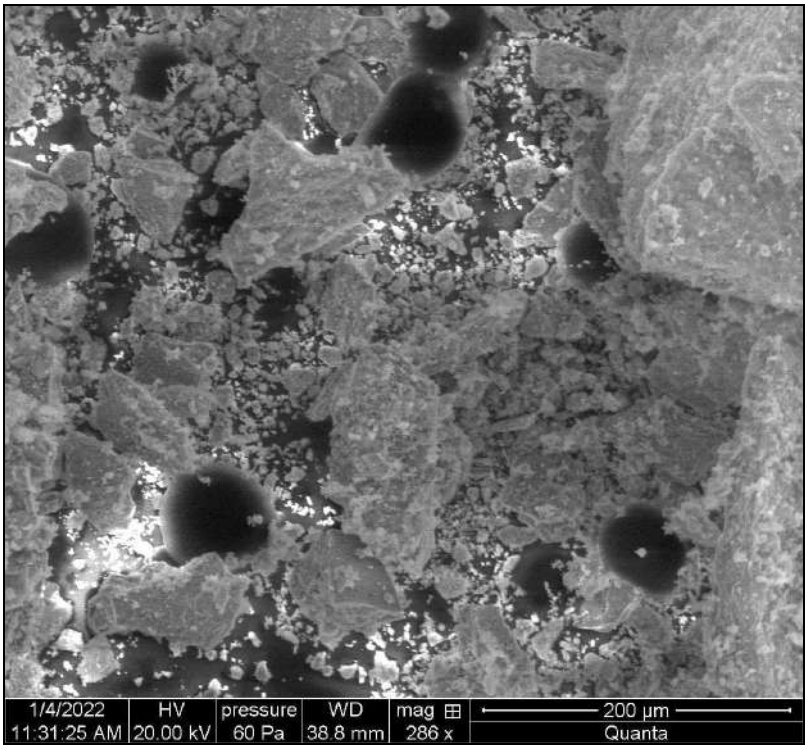

Fig 3: SEM image of loaded zinc oxide on garlic extract

Fig 2.shows the Scanning Electron Microscope image of zinc oxide prepared using zinc sulphate as the starting material and Poly Vinyl Alcohol as the surfactant. Here the negative impact of particle agglomeration and particle separation was sorted out to an extend due to the introduction of Poly Vinyl Alcohol as surfactant. Here the particle agglomeration is very less compared to the previous methods. Fig 3 Shows the SEM images at higher magnification and we can see that particles with size less than 100nm were formed also it gave clear idea about the particle separation, we can see that the particles are separated smoothly and not highly affected by agglomeration.

2. Studying the effect of the hybrid compound (garlic extract loaded with zinc oxide nanoparticles) on the viability of the sarcocyst parasite *In vitro*

Table 1: Percentage of sarcocyst parasite viability when exposed to different concentrations of the hybrid nanoparticles and for different periods of time *in vitro*

Time / Conc.	0	24h	48h	72h	96h	120h
Control	97	94.15	91.41	87.43	80.25	73.67
0.1) mg/ml)	94	59.26	48.37	36.26	26.13	17.66
0.3) mg/ml)	92	45.14	30.51	21.48	11.87	8.98
0.5) mg/ml)	90	35.13	25.36	15.12	2.9	0

L.S.D = 10.52

Table No. (1) Shows the effect of different concentrations of the nanoparticle loaded on garlic extract on the parasite's vitality for certain periods of time in the laboratory.

It was observed from the results in the table (1) there was a significant decrease of $p \geq 0.05$ in the viability of all study groups from zero to fifth day compared to the control. the higher concentration (0.5 mg/ml) was more effective in the parasite's vitality which reach (0) in the 120 hour, followed by the concentration (0.3 mg/ml) (8.98%) and the concentration (0.1) had the least effect on the parasite's vitality, because the nanomaterial has the ability to Penetrating the plasma membrane of pathogens cells and destroying them due to their small nano-size, as well as the fact that garlic is one of the medicinal plants that has great efficacy on microorganisms in a very large proportion. As well as the NPs (e.g., titanium, silver and zinc oxide) can damage and destroy the cellular components of the pathogens irreversibly e.g., membrane, DNA, protein and mitochondria, resulting in cell death (Arezou *et al*., 2018) [2].

Discussion

The present study was found that the loaded garlic extract exerts a strong and anti parasitic activity on the viability of *Sarcocystis*, where the mortality rate reached 100% at a concentration of (0.5) within 120 hours, (0.5) Within 120 hours, garlic extract's has the ability and efficacy against parasites is attributed to its allicin content. Allicin is a natural sulfur-containing compound that has many different biological properties and is responsible for the typical smell and taste of fresh cut or crushed garlic. The general perception, though not entirely accurate, of natural products as being mild and largely harmless compared to their chemically manufactured counterparts, has been suggested as one of the reasons for their increased consumer preference, as well as their increasingly common use in medicine and agriculture. Cavallito and Bailey (in 1947) clearly demonstrated that allicin is almost exclusively responsible for the antibiotic properties of garlic, and the question of the mechanism of aros and allicin's antibiotic activity. The fact that the compound possesses antimicrobial activity depends on two main features. First, the compound must be able to reach the potential targets, and if it is

intracellular, this means that it must be able to reach the inside of the microbial cell. Those are the targets of the cell. In a radical study, and using reasoning that has stood the test of time, Cavallito and his colleagues (in 1947) studied the chemistry of several antimicrobial plant compounds. It has been found that the active ingredients of Allium sativum and Erythronium americanum-Asarum reflex Arctium minus ranunculus acris Ranunculus bulbosus and Brassica species as well as non-plant-derived antibiotics, penicillin, citrinin, gliotoxin, clavasin, and pyocyanin interact with cysteine.

Conclusion

According to the results of this study, we concluded the following:

1. The process of loading the garlic extracts on the zinc oxide nanoparticles was succeed.
2. The hyprid composite has highly effect on the viability of sporocystis.
3. Garlic extract considers as efficient antiparasitic especialy when loading on zinc oxide nanoparticles.

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