World of Science: Journal on Modern Research Methodologies

Volume 2 Issue 12, Year 2023
ISSN: 2835-3072
https://univerpubl.com/index.php/woscience

Efficacy Of *Annona Squamosa* Extract Loaded on Silver Nanoparticles Against Echinococcus *Granulosus*

Sawsan Darweesh Jari

Article Information

Received: Oct 01, 2023

Accepted: Nov 02, 2023

Published: Dec 14, 2023

Keywords: *Annona squamata, Silver nanoparticles, Echinococcus granulosus, protoscolexs*

ABSTRACT

The current study was aimed to determine the Efficacy of Annona squamosa extract loaded with silver nanoparticles against the protoscolex of Echinococcus *granulosus in* vitro. The study samples were divided into four groups, The experimental groups consisted of the control group, the group treated with the unbound plant extract, the group treated with the unbound silver nanoparticles, and the group treated with the plant extract bound to the silver nanoparticles. The result showed that at zero hour, the percentage of protoscolex vitality was (97 ,92 ,90) at the concentrations (0.2 ,0.4,0.6 mg/ml) for *Annona squamata* extract, free silver nanoparticles and silver nanoparticles loaded with *Annona squamata* extract respectively. The silver nanoparticles when loaded with *Annona squamata*extract was the most effective in reducing the viability of protoscoles which reach (0) % after (120) hours in concentration 0.6 mg/ml compared to the rest of the concentrations used in the study and control group, followed by free silver treatment group which reach (8.78) after 120 hours in concentration 0.4 mg/ml compare with (18.9%) for treatment with plant extract in the fifth day. The study concluded that all treatments were affected and had significant differences in reducing the vitality percentage under 0< 0.05. but the silver nanoparticles when loaded with *Annona squamata extract* was more sufficient in reducing the vitality of *Echinococcus granulosus* protoscolex in vitro .

Introduction

Hydatid cystic disease, also known as Echinococcosis, is widely recognized as a significant global health and epidemiological issue (Zhang et al., 2017). It is an old illness that affects both people and animals. Zoonotic disease, as this disease is considered an endemic disease in some countries. Such as Iraq, Syria, Lebanon, Palestine, North Africa, Sudan, and some countries of South America (Pradhan *etal*., 2017).

Hydatid cyst disease is one of the widespread diseases in Iraq, which affects large numbers, especially in rural areas. This is due to the spread of livestock, sheep, and stray dogs, as it is considered a suitable environment for the completion of the life cycle of the parasite, which needs an intermediate host (herbivores) and a final host (carnivores). In addition to the lack of health awareness among residents of rural areas (Hayajneh *etal*., 2014).

This illness is classified as a significant health issue that poses a danger to human life and has both social and economic consequences, which has prompted researchers to find ways to reach results or solutions that reduce the effects of the disease and its spread. Also, this disease is very similar to cancer cells in the metastasis stage Metastaisis (Eckert & Deplazes, 2004).), as the disease spreads throughout the body except the hair and nails, and this occurs in advanced cases

of the disease or when the infection is caused by alveolar hydatid cysts.

The multiplicity of substances used to treat the disease chemically were partially successful, which led researchers to devise many ideas to activate the drug treatment process. Some of these researchers worked to activate non-specific immunity in the infected host by using active substances isolated from various sources such as bacteria Nunnari *etal*., (2012), fungi and plant extracts (Mahmoud, 1999; Al-Khuzai, 2005).

Others tried to develop chemical treatments by mixing drugs with each other and with other substances for the purpose of strengthening them and increasing the process of Absorption by the body, which leads to an increase in its concentration in the blood and its effectiveness against the parasite (Al-Takriti, 2004).

Medicinal plants are widely used all over the world, as they are rich in secondary metabolic products. They have been used to treat many diseases that affect humans and animals alike, in addition to not causing harmful side effects that affect the body. Among the most important effective substances are Found in medicinal plants are alkaloids, glycosides, phenols, sapanins, lectins, tannins, flavinoids, essential oils, and many other substances (Al-Khuzai, 2001).

A-Plant

B- fruit

(*Annona squamosa*)

The Annona squamosa plant is classified as a little, intricately-branched tree or shrub that belongs to the Squamosa family. It is a tropical plant that grows in the tropical lowlands of Latin America and the Euphorbia Indies and belongs to the Squamosa family. This plant was called the shelled cream plant because the external appearance of the fruits of this plant, called the Indian quince, closely resembles shells. These plants bear edible fruits called the Indian quince or the cream plant. The Annona squamosa plant is considered the most tolerant species in tropical environments, compared to other species of the same genus, which is Annona reticulate. Furthermore, Annona cherimola is characterized by its fruits, which share the same name. Therefore, Annona squamosa is grown more frequently than other species (Crane *etal*., 2016).

Active ingredients in *Annona squamosa* L.

Alkaloids are common compounds in the Annona squamosa L. plant, such as the alkaloid compound Atisine, which is found in abundance in the roots. According to Crane et al. (2016), various compounds can be found in the soursop plant, such as oxophoebine, reticuline, isocorydine, methylcorydaldine, and the flavonoid quercetin-3-O-glucoside.

Material & Methods

Prepare the solutions for the experiment:

1. Normal Saline Solution

It was prepared using the following materials:

1. Sodium chloride. Na cl 9 gm
2. Distilled water 100.

I dissolve sodium chloride in an amount of distilled water and then sterilize it using an autoclave (YX-280B) at a temperature of (121) C for half an hour and under a pressure of (15) pounds/ang2 and store it in the refrigerator at (4) C. The suspended protoscolex was collected by washing the wall of the hydatid cyst in this particular case.

2. Kerbs–Ringer Solution

This solution was prepared following the methodology of Rotunno et al. (1974). By utilizing the subsequent resources:

1. Penicillin 4000 I.U. Penicillin
2. Streptomycin 200 mg. Streptomycin
3. Sodium dihydrogen phosphate 0.097 gm. NaH2Po4
4. Disodium phosphate 0.490 gm. Na2HPo4
5. Magnesium sulfate 0.072 gm. MgSo4
6. Acid sodium carbonate 0.281 gm. NaHCo3
7. Potassium chloride 0.157 gm. KCl
8. Sodium chloride 0.480 gm. NaCl
9. Calcium chloride 0.0137 gm. CaCl2

The aforementioned substances were dissolved in a volume of one liter of distilled water. Material is only introduced when the preceding material is dissolved. The solution is sterilized by being placed in the autoclave at a temperature of (121) C while maintaining a pressure of (15) pounds/ang2 for a duration of thirty minutes. After sterilization using fine-hole filter papers (0.22 microns), glucose (0.090) grams was introduced, followed by the addition of antibiotics to prevent any potential contamination. The vitality of the protoscolex was preserved by using a certain volume of hydatid cyst fluid (HCF) in a ratio of 1:4.

3. Phosphate Buffer Saline Solution(PBS)

It was prepared using the following materials:-

Potassium dihydrogen phosphate 0.2 gm. KH2Po4

Aqueous disodium phosphate 2.89 gm. Na2 HPo4.12H2o

Potassium chloride 0.2 gm. KCl

Sodium chloride 8 gm. NaCl

Distilled water.

The above materials were dissolved in a certain volume of distilled water and then the volume was completed to (1) liter, then sterilize the solution in the incubator at a temperature of (121) lb/ang2 for half an hour (Hudson & Hay, 1989). This solution was used to wash the germinated layer.

Collecting hydatid cyst models and preparing the protoscolex

Samples of infected sheep livers were collected from a slaughterhouse in Kerbala governorate and placed inside clean nylon bags. They were then placed inside a clean container and then transported to the laboratory. Preparation and counting procedures were carried out within a period not exceeding two hours.

The method of Cowan (1999) was adopted, where the protoscolex were collected in a sterile manner. The bag's outside was disinfected using ethyl alcohol (70%), while the needle was sanitized by heat to prevent contamination of the injection site. For this purpose, plastic medical syringes with a size of (10 milliliters) were used and approximately (75%) were withdrawn from The hydatid fluid was placed in a sterile flask with a capacity of 250 milliliters. After that, the germinated layer was extracted, placed in a sterile container, cut into small pieces, and washed with a sterile physiological salt solution containing (100 international units/ml) of procaine penicillin and (100 micrograms/ml) of streptomycin and filtered through a sterile strainer whose holes allow only the protoscolex to pass through. The filtrate was collected in sterile containers. After collecting the protoscolex, washed (3-4) times with a solution of physiological saline containing antibiotics. shook lightly each time and left for several minutes until the protoscolex settled. Then the filtrate was removed and the protoscolex that were suspended in the empty physiological saline solution were kept of antibiotics.

Collecting and identifying samples of Annona fruits

They were collected from local markets and taken to the laboratory, and then the seeds of these fruits were extracted, dried, ground with an electric grinder, and then stored in plastic bottles until used.

Crud Alkaloides Compound *Annona squamosa* fruits Extracts Preparation

The researcher used the methodology outlined by Al-Samarai (1983) to isolate the alkaloid components from the Annona squamosa plant. A Soxhlets extraction device was utilized to extract 10 grams of dry matter powder into a volume of 200 ml of 96% ethyl alcohol for a duration of 24 hours. Subsequently, the resulting substance was concentrated using a rotary evaporator. Subsequently, the resultant material was dissolved in 5 ml of ethyl alcohol, followed by the addition of 30 ml of 2% acetic acid to the alcohol extract. The ethyl alcohol was then removed using a rotary evaporator, leaving behind the acidic solution. The alkaloids were identified using the Druckendorff reagent, which produced an orange or yellowish-orange solid when tested. After combining a drop of the reagent with a drop of the acidic solution, a suitable quantity of ammonium hydroxide with an amount of 10% is added to the resulting mixture until the pH level reaches 9. The fundamental solution was introduced into a separating funnel, followed by the addition of 10 ml of chloroform. The mixture was vigorously agitated many times. The mixture was allowed to stand until it reached a state of equilibrium, resulting in the formation of two distinct layers. The underlying stratum, including the alkaloids, was extracted and this process was iterated several times to get a 40 ml solution. Subsequently, an adequate quantity of sodium sulfate was introduced. Anhydrous substances are used to remove moisture from the solution. The filtrate underwent further concentration in the rotary evaporator to facilitate the evaporation of the chloroform. Multiple iterations of the extraction procedure were conducted in order to acquire an adequate quantity of alkaloid chemicals. The desiccated alkaloid substances were kept in a glass container inside the refrigerator until they were needed.

Preparation of silver nanoparticles

Combine 0.0285 grams of sodium borohydride with 10 milliliters of deionized water in an ice-cold solution labeled as number 1.

Introduce 0.4 mg of polyvinyl pyrrolidone into the initial solution as a stabilizer to facilitate the

contact.

Combine 0.0214 grams of silver nitrate with 10 milliliters of distilled water to create Solution No. 2. Agitate the solution for 60 minutes at a temperature of 50–60 °C with a stirring speed of 1500 rpm to transform it into a dark brown hue. 6. Subsequently, the solution was allowed to cool and rinsed with distilled water. The procedure was iterated three times, subsequent to which the solution was dried in an electrical oven. The product was gathered and conserved for utilization in the medication loading procedure (Zhu et al., 2011).

Loading of the Annona plant onto silver nanoparticles

A quantity of (1.5) milligrams of Annona plant extract powder was measured and combined with (1.5) milligrams of the unbound nano-silver compound, which had been dissolved in (10) milliliters of distilled water. Subsequently, the mixture was subjected to magnetic stirring for a duration of 24 hours, followed by concentration. The sample was dehydrated using a rotary evaporator and stored for the purpose of standardizing and conducting research on the viability of protoscolex in a laboratory setting (Derayea et al., 2017).

The outer surface of the silver nanoparticles was studied before loading and after loading the plant extract using an atomic force microscope:

The roughness of this surface and its square root were calculated according to the following equation:

$$Rm = \sqrt{\sum_{i=1}^{n} \frac{(Zi - Zav)^2}{N}}$$

Standardization using scanning electron microscope (SEM)

The free silver nanoparticles compound and the silver loaded with plant extract were examined using a scanning electron microscope (SEM)for the purpose of knowing the surface shape of the two compounds under study. The samples were sent to Kashan University in Iran for the purpose of examining the models.

Study of the efficacy of the alkaloid extract of *Annona squamosa* on the vitality of protoscolex in vitro.

The effect of the alkaloid extract of the *Annona squamosa* plant on the vitality of protoscolex was studied outside the living body. The study samples were divided into four groups, The control group, group treated with the free plant extract, group treated with the free silver nanoparticles and group treated with the plant extract loaded on the silver nanoparticles.Each group consists of 20 protoscolexs placed in five petri dishes. Each plate contains Zenker's solution and hydatid fluid(4:1). Each group was treated with the following concentrations at concentrations (0.6, 0.4, 0.2) mg/ml. These concentrations were chosen based on the LD50, then the highest and lowest concentration was chosen, After that, vitality in the suspension were calculated based on Ma *etal*. (2007).

Statistical Analysis

The data was subjected to statistical analysis using SPSS version 22. The research used the least significant difference (L.S.D.) test to identify significant differences between the study groups. The probability level of 0.05 or less is denoted as P (Morgan et al., 2010).

Result and Discussion

Study of the effect of Annona extract loaded on silver nanoparticles on the vitality of protoscolex in vitro.

When using different concentrations of *Annona* fruit, we notice a difference in the vitality of the protoscolex from the zero hour until the fifth day. At zero hour, the percentage of protoscolex vitality was (97, 92, 90) at the concentrations (0.2, 0.4,0.6 mg/ml) respectively compared to the percentage of protoscolex viability on the fifth day which reached (18.9)% when exposure to plant extract (0.2mg/ml) and (8.78) % at the concentrations (0.4 mg/ml)of free silver nanoparticles. While the vitality of the protoscolex in the same day was (0) at the concentration of (0.6 mg/ml), which led to the killing all protoscolex, as shown in the table (1).

Table (1) Percentage of viability of protoscolex when exposed to different concentrations of plant extract, Free Silver nanoparticles and *Annona squamosa* loaded on silver nanoparticles and for different periods in vitro.

120	96	72	48	24	0	Time (hour) / Concentration mg/ml
74.7	81.8	92.8	94.5	94	99	Control
18.9	31.11	44.3	52.5	60.5	97	0.2 mg/ml (plant extract)
8.78	16.01	21.61	32.27	47.01	92	0.4mg/ml(free silver nanoparticles)
0	11.87	19.35	29.03	39.06	90	0.6 Mg/ml (silver loaded with plant extract)
L.S.D	7.86					

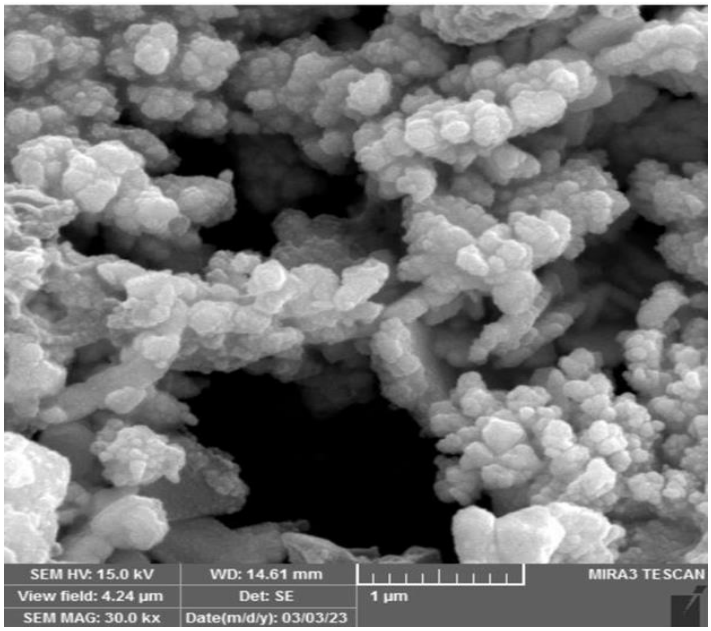
Result & Discussion

Standardization of Free & Loaded Silver Nanoparticles Using Spectroscopic Method

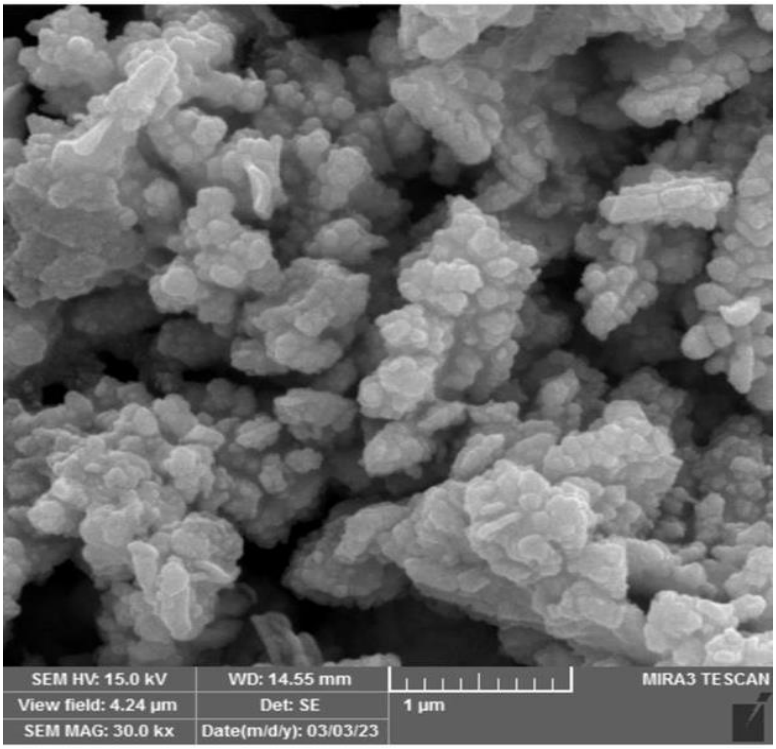
Standardization A Scanning Electron Microscope (Sem)

The outer surface of the silver nanoparticles was studied before loading and after loading the plant extract using an atomic force microscope:

The image (1) showed the outer surface of free silver nanoparticles with a scanning electron microscope(SEM).. And the image (2) for silver nanoparticles loaded with plant extract, the difference was observed before and after loading. The size of the plant extract loaded on the silver nanoparticles surfaces plays an important function in the surface roughness and its regular crystalline system as well as the surface homogeneity.



The image (1) showed the outer surface of free silver nanoparticles with a scanning electron microscope(SEM).



The image (2) showed the outer surface of free silver nanoparticles loaded with plant extract using a scanning electron microscope(SEM).

Conclusions

The study concluded that Free and loaded nanoparticles indicates the success of the synergistic action between the silver nanoparticles and plant extract upon loading and all treatments were effected and had significant differences in reducing the vitality percentage and the silver when loaded with *Annona squamata extract* was more sufficient in reducing the vitality of *Echinococcus granulosus* protoscolex in vitro.

References

1. Thang, T. D.; Dai, D.N.; Hoi, T.M. and Ogunwande, I.A. (2013). Study on the volatile oil contents of Annona glabra L., Annona squamosa L., Annona muricata L. and Annona reticulata L., from Vietnam. Natural Product Research.Formerly Natural Product Letters Volume 27, 2013.
2. Zhang, R ; Chen, X. and Wen, H. (2017). An improved experimental model of cystic hydatid disease in liver resembling natural infection route with stable growing dynamics and immune reaction. bioRxiv, 155168.
3. Pradhan, S., Dahal, R., Phuyal, S., & Ghimira, B., Singh, Y. (2017).Primary Hydatid Cyst of Pancreas. Retrieved from Case Report. Acta Scientific Medical Sciences., 101: 25-27.
4. Nunnari, G.; Pinzone, M. R.; Gruttadauria, S.; Celesia, B. M.; Madeddu, G.; Malaguarnera, G.; Pavone, P.; Cappellani, A. and Cacopardo, B. (2012). Hepatic echinococcosis: Clinical and therapeutic aspects. *World. J. Gastroenterol*.,18(13):1448-1458.
5. Morgan, G. A.; Leech, N. A.; Gloecner, G. W. and Barrett, K. C. (2010). SPSS for introductory statistic: use and interpretation. 2nd ed. Lawrence Erlbum associates, publisers Mahwah, New Jersey, London.
6. Ma, X. M.; Bao, G. SH., Wan, J. M.; Liao, D. J.; Yin, SH.F.; Meng, X. Q.; Zhou, G. K.; Lu, X. M. and Li, H. Y. (2007). Therapeutic effect of *Sophora moorcroftiana* alkaloids in

combination with albendazole in mice experimentially infected with protoscolices of *Echinococcus granulosus*. Braz. J. Med. Biol. Res., 40(10): 1463-1708.

7. Mahmoud, S. S. and Al-Izzi, M. S. (1999). The efficacy of current scolicidal agents in surgically removed hydatid cysts. Ann. Coll. Med. Mosul, 25(1-2): 91-93.
8. Rotunno, C. A.; Kammerer, W. S.; Esandi, M. and Cerejido, M. (1974). Studies on the permeability to water, Sodium and Chloride of the hydatid cyst of *E. granulosus*. J. Parasit., 60(4):613-620.
9. Derayea, S. M.; Ali, H.R.H.; Hamad, A. A. and Ali, R. (2017). Application of silver nanoparticles for the spectrophotometric determination of three benzimidazole anthelmintic drugs in their pharmaceutical preparation. Journal of Applied Pharmaceutical Science, 7 (02): 076-082.
10. Zhu, Journal Kan, C.; Wang, Journal G. & Wang, M.H. (2011). High yield of uniform synthesis Ag nano wire High Aspect Ratio by introducing the long. Chain pvp in an lmproved polyol process. *Journal of Nanomat.*, 7 :122- 133.
11. Al-Shamaa, A. A. (1989). Chemistry of medical plants. House of books for puplishing and printing.
12. Cowan, M. M. (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12(4): 564-582.
13. Hudson, L. and Hay, F. C. (1989). Practical immunology. 3rd edn. Blackwell Scientific Publication.
14. Crane, J.H.; Carlos, Balerdi,C.F.and Maguir,A. (2016). Sugar apple grawing in the florida home landscape. UF/IFAS Extension. 1658pp.
15. Al-Takriti, Kholoud Naji Rashid (2004). A preliminary study to estimate the effectiveness of of substances (plant, chemical, antigenic) against the infectiveness of the watery cyst parasite *E. graunlosus* as an attempt to use it therapeutically. Master's thesis, College of Science, Al-Mustansiriya University: 187 pages.
16. Al-Khuzai, Jassim Hamid Rahma (2005). Evaluating the effectiveness of black seed extract, some medications, and electric current on the vitality of the protoscolex of the parasite *Echinococcus granulosus*. Doctoral thesis - College of Education - Al- Qadisiyah University. 132 pages.
17. Eckert, J. and Deplazes, P. (2004). Biological, Epidemiological, and Clinical aspects of echinococcosis, a zoonosis of increasing concern. Clin. Microbiol. Rev., 17(1): 107-135.
18. Hayajneh, F. M. F., Althomali. A. M. H., & Nasr, A. T. (2014). Prevalence and characterization of hydatidosis in animals slaughtered at Al Taif abattoir, kingdom of Saudi Arabia. Open Journal of Animal Sciences, 4(10), 38.