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Evaluation of the efficiency of chitosan and silver nanoparticles in the treatment of lice experimental infestation in local chickens

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Abstract

The current study aimed to determine the effect of silver and chitosan nanoparticles of size 10 to 30 nm on the dead of lice *in vitro* and *in vivo* to determine the optimal time and concentration to combat chicken lice. One hundred local chickens *Gallus gallus* domesticus were collected from Al-Diwaniyah province and 6 species of local chicken lice were isolated: *Menacanthus stramineus, Menacanthus pallidullus, Menacanthus cornutus, Goniodes gigas, Cuclotogaster heterographus* and *Bonomiella columbae*. The results of treating lice with chitosan and silver nanoparticles at concentrations (40, 60, and 80 mg/mL) *in vitro* and at different periods (5, 10, 15, and 30 minutes) after treatment showed that chitosan and silver nanoparticles at a concentration of 80 mg/mL are the most effective in killing lice. The dead rate of lice reached 100% after 15 minutes of treatment with chitosan nanoparticles and 100% in the case of silver nanoparticles after 30 minutes. The results of spraying chitosan and silver nanoparticles on the body of chickens infected with lice experimentally, based on the relative therapeutic efficacy within 30 minutes, indicated that silver nanoparticles were the most effective in completely killing lice in the group treated with a concentration of 80 mg/kg after 30 minutes, where the percentage of therapeutic efficacy was 96.7%. This was followed by chitosan nanoparticles at a concentration of 80 mg/kg, and the percentage of therapeutic efficiency was 91.5%. Chitosan and silver nanocomposite have a promising effect in the elimination of lice infestation in chickens.

Keywords: chitosan; sliver; nanoparticles; Lice; Iraq

Introduction

Due to the resistance shown by many insects towards chemical pesticides due to excessive use, it has been observed in recent years a great trend towards nanomedicine to combat parasites of various species [1], as it has been proven that nanoparticles (NPs) can be used to treat diseases of various species may be bacterial, viral, fungal or parasitic [2]. Some materials, when reduced to nano size, undergo a change in their properties, leading to the possibility of using them on a large scale to monitor, diagnose, treat and combat infectious diseases [3]. NPs have well-defined chemical, mechanical, and optical properties and one of their properties is their cytotoxic effect, which varies with size, shape, charge, purity, and stability [4].

Chitosan is a polysaccharide polymer derived from chitin and produced by removing the acetate portion of it, it is extracted from the shells of crustaceans such as crabs as well as from the cell walls of fungi, it is a biodegradable and biocompatible polymer that is considered safe for human food use and is approved for wound dressing. It has been approved by Approved by the US Food and Drug Administration for tissue engineering and drug delivery [5], and recent research indicates its use as a pesticide for external parasites such as the hard tick parasitic on livestock [6], and in another study, Attia et al. [7] indicated that spraying chitosan NPs on pigeons infected with the fly *Pseudolynchia canariensis* in cages for 15 minutes gave a promising result as an anti-parasitic agent as well as healing the wound resulting from the insect bite. Silver NPs are used in various fields such as medicine, food, healthcare, consumer and industrial purposes [8–10], and due to their physical and chemical properties, they have been used in many applications, including antibacterial agents. In diagnostics, orthopedics, wound dressings, biomedical devices, drug delivery, and as anticancer agents [11–13].

Silver NPs, AgNPs, were used as an alternative for the treatment and control of the red chicken mite *Dermanyssus gallinae* [14]. AgNPs extracted from *Euphorbia hirta* leaves were tested against *Anopheles stephensi*, and these molecules showed high larval killing activity [15]. AgNPs synthesized from the fungal species *Chrysosporium keratinophilum* and *Verticillium lecanii* also showed good activity against pupae and larvae of *A. stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti* [16]. In another study, Attia et al. [7] concluded that spraying pigeons experimentally infected with the *P. canariensis* fly with low concentrations of silver NPs had an effective role in controlling most of the flies, as it acted as an effective biopesticide.

Materials and Methods

Ethical approval

The Committee of the Department of Biology, Faculty of Education, University of AL-Qadisiyah approved this study (No. 986).

Isolation of lice samples

During the period from July 2023 to March 2024, 100 local chickens *Gallus gallus* domesticus were collected from Al-Diwaniyah province, and 6 species of biting lice were identified, *Menacanthus stramineus, Menacanthus pallidullus, Menacanthus cornutus, Cuclotogaster heterographus, Bonomiella columbae*, and *Goniodes gigas*, its diagnosed based on [17].

Characteristics of nanoparticles used in the study

The nanocomposites were purchased in the form of ready-made silver NPs from one of the accredited American companies, Sky Spring Nanoparticles Inc., and the chitosan belongs to a Canadian company, Nanochemazone Inc., in powder form, with a purity of 99.8% and a size of 10 to 30 nanometers for both chitosan and silver NPs. Ag NPs appearance dark grey powder while chitosan NPs were white in color (Fig. 1).

Preparation of different concentrations of chitosan and silver nanocomposites

A stock solution was prepared from the 2 nanocomposites, chitosan and silver, by dissolving 1 g of each separately in 200 mL of distilled water for each one. The solutions were sterilized with an autoclave, then each solution was mixed using an ultrasonic homogenizer for 15 minutes, then 3 concentrations were

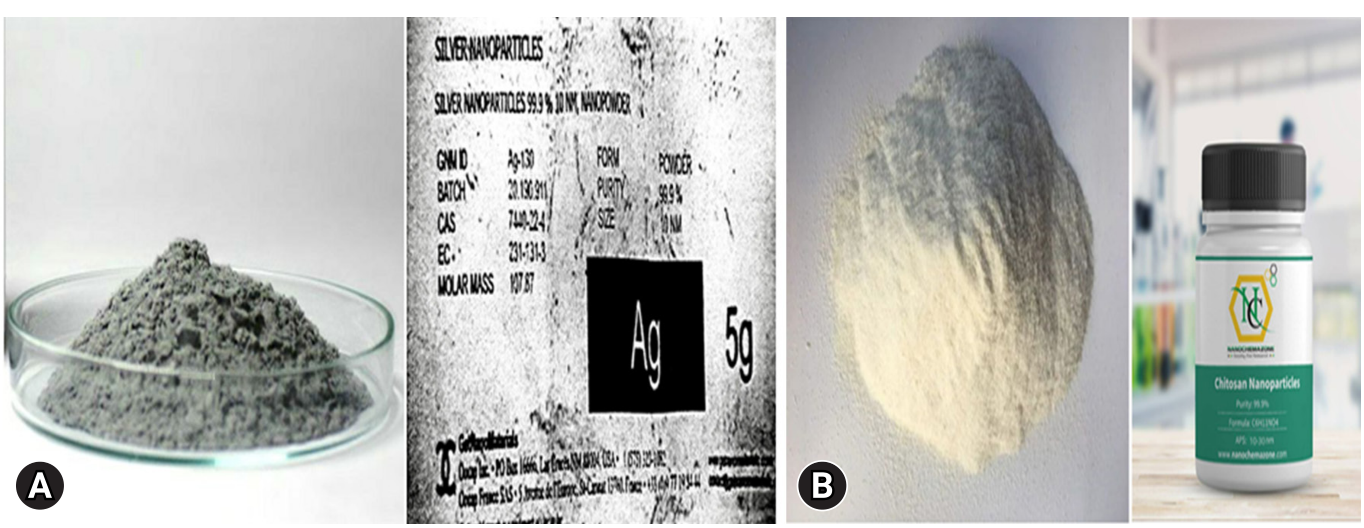

Fig. 1. (A) Silver nanoparticle. (B) Chitosan nanoparticles.

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prepared for each one 40, 60, and 80 mg/mL and kept in the refrigerator until used in the experiment [18].

Experimental and laboratory study

Role concentrations of chitosan and silver nanoparticles on the mortality rates on lice in vitro

This experiment was conducted to test the effectiveness of 3 different concentrations of 40, 60, and 80 mg/mL for each of the chitosan and silver NPs on lice parasitizing chickens *in vitro*. Seven Petri dishes were used and an equal number of lice (8) were placed in each dish, the first dish was treated with a concentration of 40 mg/mL of ChNPs, the 2nd dish was treated with a concentration of 60 mg/mL of ChNPs, the third dish was treated with a concentration of 80 mg/mL of ChNPs, dish 4 was treated with a concentration of 40 mg/mL of AgNPs, and dish 5 was treated with a concentration of 60 mg/mL of AgNPs, while dish 6 was treated with a concentration of 80 mg/mL of AgNPs, while plate 7 was left as a control group. After that, the dishes were observed under a microscope for 3 replicates during different times (5, 10, 15, and 30) minutes, in order to determine the numbers of live and dead lice. After that, the optimal time for the effectiveness of this substance with its different concentrations against lice parasitizing on chickens was determined.

Role concentrations of chitosan and silver nanoparticles on the mortality rates on lice in vivo

For the purpose of conducting an experimental infection with biting lice in local chickens, 42 local chickens were used in the experiment. They were confirmed to be free of external and internal parasitic infections through clinical examination and microscopic examination of the feces for a week. They were placed in cages, each cage 1 m long, 0.5 m wide, and 1 m high. Cover the floor with sawdust approximately 4 cm thick. Then the birds were distributed into 10 groups, each containing 3 birds, with the birds being given the final feed. As for water, it is provided in plastic manholes with a capacity of 1 liter, and the experiment was designed as follows: (1) 30 uninfected chickens were taken and placed in special breeding cages prepared for this purpose and described above. (2) The birds were divided into 10 groups (6 experimental groups) and 2 control groups for each one nanomaterial separately (positive and negative), each one containing 3 chickens, and left for a week under control and in ideal conditions. (3) Two naturally infected chickens with heavy lice infestation were placed with each of the experimental groups and left for 2 weeks, anesthetize the chickens using chloroform in very low doses by inhalation and in an open atmosphere in order for the chickens to remain alive and in order to calculate the therapeutic efficiency of chitosan and silver NPs, based on Abbott. (4) The efficiency of NP concentrations of 40, 60, and 80 mg/kg of chitosan compound in eliminating lice was tested by spraying the groups as follows: the first group, G1, with a concentration of 40 mg/kg of chitosan NPs; second group, G2, with a concentration of 60 mg/kg of chitosan NPs; third group, G3, with a concentration of 80 mg/kg of chitosan NP; the fourth group, G4, was left as a positive (infected) group without any treatment; the fifth group, G5, was left as a negative group (uninfected). (5) As for silver NPs, concentrations of 40, 60, and 80 mg/kg were tested by spraying the following groups: the first group, G1, with a concentration of 40 mg/kg of silver NPs; the second group, G2, with a concentration of 60 mg/kg of silver NPs. (6) The third group, G3, with a concentration of 80 mg/kg of silver NPs; the fourth group, G4, was left as a positive (infected) group without any treatment; the fifth group, G5, was left as a negative group (uninfected(.

Solutions of chitosan and silver NPs were sprayed on the areas of the body most affected (butt, Fig. 2) by the concentrations mentioned above, and the feathers were examined after different times of treatment for each concentration, focusing on the most affected area to calculate the rates of infestation of live and dead lice.

Statistical analysis

A completely randomized design was used, with a factorial experiment with 2 factors (concentrations and time periods). The results were tested according to the least significant differences test and at the probability level of $p < 0.05$.

Results

Effect of different concentrations of chitosan and silver nanoparticles on lice mortality rates *in vitro*

The results showed, as shown in Table 1, an increase in the

Fig. 2. Chicken butt contain different species of lice (arrow).

Table 1. Effect of different concentrations of chitosan and sliver nanoparticles on lice mortality rates *in vitro*

Nanoparticle	Concentration (mg/mL)	5 min (n = 8)	10 min (n = 8)	15 min (n = 8)	30 min (n = 8)
Chitosan	40	1 (12.5)	1 (12.5)	4 (50.0)	5 (62.5)
Chitosan	60	1 (12.5)	3 (37.5)	5 (62.5)	6 (75.0)
Chitosan	80	4 (50.0)	5 (62.5)	8 (100.0)	8 (100.0)
Sliver	40	1 (12.5)	3 (37.5)	4 (50.0)	6 (75.0)
Sliver	60	3 (37.5)	4 (50.0)	6 (75.0)	7 (87.5)
Sliver	80	4 (50.0)	4 (50.0)	7 (87.5)	8 (100.0)
Control groups	0	0 (0)	0 (0)	0 (0)	0 (0)

Values are presented as number (%).

rate of lice dead with increasing concentration and duration of exposure to the chitosan and silver NPs, where the rate of lice dead at concentrations of 40, 60, and 80 mg/mL of chitosan NPs after 30 minutes of treatment reached 62.5%, 75.0%, and 100.0% respectively, which represents the highest percentage of lice dead compared to the percentage of dead of lice treated with the same concentrations and shorter periods of time, while the lowest percentage of dead was in the group treated 5 minutes after treatment, as it reached 12.5%, 12.5%, and 50.0% respectively,. The dead rate increased proportionally with time, as the dead rate after 15 minutes reached 50.0%, 62.5%, and 100.0% respectively, followed by the dead rate of lice after 10 minutes of treatment, where the dead rate reached 12.5%, 37.5%, and 62.5% respectively.As for silver NPs, it was observed that the rate of lice dead at concentrations of 40, 60, and 80 mg/mL of silver NPs after 30 minutes of treatment was the best compared to the rest of the other times, as the rate of lice dead reached 75.0%, 87.5%, and 100.0%. The lowest percentage it was also in the treated group after 5 minutes, as the rate of lice dead reached 12.5%, 37.5%, and 50.0%.

Effect of different concentrations of chitosan and sliver nanoparticles on the rate of lice mortality and the relative therapeutic efficacy of chickens experimentally infected with lice *in vivo*

The NPs were sprayed on the body of the infected chickens experimentally, and the most affected area of the body was chosen, which was the butt for the purpose of calculating the relative therapeutic efficiency of the NPs within 30 minutes.

The results of the current study, as shown in (Table 2), indicated that the average number of lice in infected chickens treated with chitosan NPs at 3 concentrations of 40, 60, and 80 mg/kg was less than the average number of lice in infected untreated chickens (positive control), with a statistically significant difference at a significance level of $p < 0.05$. The results of the therapeutic efficiency of chitosan NPs after 30 minutes reached 33%, 66.5%, and 91.5%, respectively. The study showed that the best concentration that led to a reduction in the infection rate in chickens was 80 mg/kg. It is also clear that spraying silver NPs on infected and treated chickens gave valuable results, as it led to a significant reduction in the number of lice, especially at the concentration of 80 mg/kg compared to the negative control group. As for the results of the therapeutic efficiency of the 3 concentrations after 30 minutes, it reached 43.4%. 73.4%, and 96.7%, respectively. The results also showed that silver NPs at a concentration of 80 mg/kg were sufficient to kill lice within 30 minutes.

Discussion

Parasites of both external and internal species are treated with anti-parasitic chemical treatments, but over time, resistance to these medications has arisen due to excessive use, while recent years have witnessed tremendous development in the field of nanomedicine to combat parasites, as some NPs have shown promising results in treating various species of parasitic infections [1], where scientists have proven that NPs can be used to treat bacterial, viral, fungal, and parasitic diseases [2].

It is clear from the results of the current study that chitosan and silver NPs have a clear effect on the dead of lice *in vitro*, and this effect is directly proportional to the increase in concentration and duration of exposure to these particles. The rate of lice dead differed among them depending on the type of nanomaterial used, as the highest rate of lice dead reached within 15 and 30 minutes using 80 mg/mL of chitosan NPs at a rate of 100%, while the percentage of lice killed using silver NPs within 15 minutes was 88%, and the complete killing rate of 100% was achieved within 30 minutes. These results are similar to what some researchers have achieved. In one study, Sioutas et al. [14] used AgNPs as an alternative for treatment and control of the red chicken mite *D. gallinae*, On the other hand, Barbosa et al. [6] indicated in his experiment on Rhipicephalus ticks, which

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Table 2. Effect of different concentrations of chitosan and sliver nanoparticles on the rate of lice mortality and the relative therapeutic efficacy of chickens experimentally infected with lice *in vivo* at 30 minute

Type of group	Nanoparticles (mg/kg)	Rate no. of live lice	Rate no. of dead lice	Relative therapeutic efficacy (%)
G1	Chitosan 40	8	4	33
G2	Chitosan 60	4	8	66.5
G3	Chitosan 80	1	11	91.5
G4	Positive (infected) group	12	0	-
G5	Negative (uninfected)	0	0	-
G1	Silver 40	5.66	6.34	43.4
G2	Silver 60	2.66	9.34	73.4
G3	Silver 80	0.33	11.67	96.7
G4	Positive (infected) group	12	0	-
G5	Negative (uninfected) group	0	0	-

-, The groups are not included in the treatment.

included 3 different concentrations of chitosan NPs, the ability of these particles to reduce the mass of female tick eggs, Abu-Elala et al. [19] also observed that the chitosan-silver nanocomposite was effective in treating *Lernaea cyprinacea* infection in goldfish aquariums, Gaafar et al. [20] also demonstrated that chitosan-silver NPs have an effect against *Toxoplasma* in Swiss albino mice. In addition, many researchers have confirmed that the chitosan-silver nanocomposite has a powerful and destructive antibacterial effect, especially against *Escherichia coli* and *Salmonella typhimurium* [21].

After creating an experimental infestation with lice in local chickens, chitosan and silver NPs were sprayed on the chicken's feathers, focusing on the butt area because it is the most infested area and for easy observation of dead lice because it contains few feathers. This is to calculate the therapeutic efficiency of both materials, as the ideal time was determined as 30 minutes based on this. In the first experiment, which was outside the living body, the effect of the particles used in killing lice varied.

Silver NPs were the most efficient in completely killing lice in the treated group at a concentration of 80 mg/kg after 30 minutes, where the percentage of therapeutic efficiency was 96.7%, followed by chitosan NPs also at a concentration of 80 mg/kg, and the percentage of therapeutic efficiency was 91.5%. These studies are consistent with other studies conducted on other ectoparasites, such as ticks, as Barbosa et al. [6] indicated the possibility of using chitosan NPs as an acaricide in livestock. In another study, Attia et al. [7] concluded that spraying pigeons experimentally infected with the *P. canariensis* fly with low concentrations of silver and chitosan NPs had an effective role in controlling most of the flies, as it acted as an effective biopesticide. The dead of lice due to nanomaterials can be attributed to the effect of the nervous and digestive system of the insect upon contact with the chitosan and silver NPs, or it may lead to the suffocation of the insect as a result of their entry through the breathing holes or because these NPs come into direct contact with the insect's body. In this study, nanotechnology opens new facilities for control from the lice. Spray the chicken with nanocomposites of chitosan and silver in low concentrations more effective role to control lice Acts as a powerful biocide. Apply as a spray of chitosan and silver nanocomposites at a concentration of 80 mg/kg on chickens in cages for 30 minutes gave promising results an anti-parasitic agent as well as wound healing caused by an insect bite, with a slight superiority of silver NPs over chitosan.

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