THE BIOCHEMICAL EFFECT OF BIOLOGICALLY PREPARED SILVER NANOPARTICLES ON CHICKENS EXPERIMENTALLY INFECTED WITH THE CYST PARASITE EIMERIA TENELLA

Khalaf Hasan Taresh¹, Shehab Ahmmd Mohammed²
E-mail: khalfiassan.1988@gmail.com

ABSTRACT

The current study aimed at the effect of silver particles on the mortality rate, the weight of chicken chicks, the amount of feed consumed, the expulsion of egg bags with feces, and the hematological and biochemical effect on chickens infected with an experimental parasite, Eimeria tenella, M., where the cocci parasite intruding on the naturally infected Ross foreign chickens was investigated and the parasite was isolated from the cases received to the veterinary hospital and veterinary clinics from the poultry fields, and then it was initially diagnosed by direct microscopic examination with a wet swab of feces, and then the diagnosis of the sample was confirmed in the veterinary hospital / Salah. The size of the sporulated egg bag was 21 micrometers in length and 19 micrometers in width, then the challenge strain was obtained by strengthening the isolation and its effect on Ross 308 broiler chickens, then the lowest dose of mature egg bags was obtained to cause 50% mortality in chicks. The fermented was 50,000 mature egg sacks.

The results of the experiment showed that the experimental infection with the type E.tenella was shown in the chicks of foreign Ross chickens, which included the symptoms and clinical signs. Clinical signs were observed, such as a state of lethargy, loss of appetite and irregular feathers, as well as bloody diarrhea, weight loss, wasting and isolation. As for the pathological changes Otitis media was represented by congestion of the cecum and an increase in their size. Within the cecum, extensive ulcers were observed and the cecum was filled with blood, mucous and fluids. The results of the experiment showed that the experimental infection with the type E.tenella (a highly significant \( P < 0.05 \)) decrease in the number of egg sacks presented in The faeces of the chicks that were treated with silver nanoparticles compared to the positive control, and a highly significant \( P < 0.05 \) decrease in the mortality rate was observed in the chicks that were treated with silver nanoparticles compared to the wave control. It was treated with secondary silver particles, and the results showed a highly significant \( P < 0.05 \) In the value of the blood images represented by the size of the pcv cells and the level of hemoglobin (hemoglobin) and red blood cells in the chicks that were treated with silver nanoparticles compared with the positive control, the results also showed a highly significant \( P < 0.05 \) increase in the level of the biochemical characteristics of the blood serum represented by The level of glucose, cholesterol, triglycerides, calcium and albumin in chicks treated with silver nanoparticles compared to the positive control.

Keywords: Eimeria tenella biologically prepared silver nanoparticles.

I. INTRODUCTION

Coccidiosis is one of the most dangerous parasitic diseases that affect poultry, not only in Iraq, but in the Arab world and throughout the world. Despite the scientific progress that has taken place in the field of prevention and treatment of diseases in general, it remains the first parasitic enemy facing the poultry industry because It is caused by health problems that lead to huge economic losses represented in the loss of income and the cost of treatment, prevention and control of pollution as well as the high fatalities it causes among poultry flocks .It is caused by a species of parasitic protozoan belonging to the family Eimeriida, which includes several genera, including genus Eimeria. (Jadhav et al., 2011).

Infection with Eimeria tenella is one of the biggest problems faced by poultry breeders (broiler chickens, laying hens) in the world, as well as one of the most dangerous and virulent types of Eimeria, as it lives and multiplies in
the cecum, causing destruction in the intestinal cells, which interferes with the absorption of indigestible materials and causes dehydration of the body and blood loss (Adhikart et al., 2008).

The weak immune response of modern breeds of broilers and broilers made them vulnerable to continuous diseases and deaths, and the problem of the spread and treatment of diseases became one of the most important problems facing the expansion of poultry farming (EL.Behairy et al., 2005). It seems that the attempts shown by the pharmaceutical industry to develop a new generation of anticoccidiosis has made the process of challenging the phenomenon of resistance shown by Eimeria species impose on researchers many scientific efforts to search for alternative ways to control coccidiosis (Lee et al., 2009a) and chemotherapeutic and preventive chemical drugs. (Raja et al., 2009).

Humanity has gone through many scientific revolutions and progress in the fields of life, and in the last two decades it has witnessed the beginning of the fifth scientific revolution, which is nanotechnology. Scientific applications that produce materials by assembling them at the small level from their basic components, and the word nano is derived from the Greek word dwarf or small Nanos or Dwarf in English (Jaison et al., 2018) and it becomes clear to us that nanoscience is the science that deals with the study and characterization of nano materials as well On the knowledge of their physical and chemical properties and the study of the phenomena associated with reducing their size, as reducing the sizes and measurements of materials to nanometers is not a goal, but rather a scientific philosophy and a qualitative and scientific coup against physical and chemical theories to produce new materials known as nanomaterials (Sau and Rogach, 2010).

II. MATERIALS AND METHODS

2.1 Parasite isolation

2.1.1 Parasite specimen collection

2.1.2 Diagnosis oocyst of Eimeria tenella

Isolation was diagnosed by relying on the gross examination of the pests and the site of intrusion and the microscopic examination of the egg sacs in terms of shapes and dimensions according to (Saif et al., 2008). The dimensions of E.tenella Oocyst were measured, using a microscope and Graduated Ouliar Micrometer, as well as using a stage Micrometer. The slide was placed on the platform of the compound microscope and the zero scale in both the lens and the glass slide was perfectly matched and the value of each two identical lines in the lens and slide was recorded, then The average was extracted for three readings , and to obtain the microscope parameter, the following equation was used:

\[
\text{microscope parameter} = \frac{\text{Microscope measurement rate}}{\text{Ocular meter reading rate}} \times 100 \text{ micro letter}
\]

Actual dimensions of the egg sacs = Measurement of the egg sacs distances using the included eyepiece × Microscope parameter.

The stage micrometer slide is dispensed with and only the ocular micrometer lens is used to make the measurements. The diagnosis of the isolation of E. tenella Oocysts cysts was confirmed in the Veterinary Hospital / Department of Poultry Diseases, Salah al-Din.

2.1.3Preparing the bags of ripe oocyst.

Egg bags were collected by taking the Caecum(with their contents), and they were placed in an electric mixer, then the mixture was taken and filtered by a piece of tulle, then filtered through a strainer (sieve) 50 mesh/cm to rid of the suspended residues, the mixture was precipitated by centrifugation quickly 3000 min cycles for 5 minutes, the precipitate was washed with distilled water and precipitated again (the method was repeated several times), then two methods were used to obtain the oocysts:

1. Sedimentation: After washing the precipitate several times, a sample was taken and examined under a microscope to confirm the presence of infection.

2. Floatation: It was by two methods:

a) by saturated salt solution NaCl.
b) by Sheather's solution (Bowman and Lynn, 1995).

the percentage of infection was extracted by using the following equation:

\[
\text{percentage of infection} \% = \frac{\text{number of infected samples}}{\text{total number of samples}} \times 100
\]

After flotation of 2 g of the precipitate with 15 ml of Scheither's sugar solution or saturated saline solution (the flotation process was repeated three times) and each time the filtrate was placed with its predecessor in a clean beaker, the filtrate was taken and diluted with distilled water in a ratio of 1 leach: 10 distilled water, then used. Centrifuge the sample at 3000 rpm for 5 minutes. The total precipitate was taken and placed with an equal amount of 5% potassium dichromate solution (K\(_{2}\text{CrO}_7\)) to preserve the oocysts. The total solution was placed in a clean, sterile glass beaker and placed in a shaker incubator at room temperature. 25-28 \(\text{m} \) for 72 hours and cover the flask with sterile perforated silver paper that allows the entry of oxygen necessary for the process of spores to occur (Bowman and Lynn, 1995) and fill the solution with distilled water from time to time so that the solution does not dry out (1955, Edgar), it was ascertained the maturity of the oocysts, by examining them under the microscope, took a drop of the suspension and placed it on a glass slide where it was covered with the cover of the slide and examined under the light microscope to observe the egg sacs containing the spore sacs that contain the spores inside and the stieda body, which appears clearly in the oocyst when the completion of the process of sporulation (Shiotani, 1992).

2.2.4 Purification of Oocysts

The parasite Oocysts were purified based on what was stated by Jeffers (1978). The suspension was washed with distilled water to get rid of potassium dichromate solution using the sedimentation method by Centrifuge at 3000 rpm for five consecutive times for 5 minutes for one time, then put. The precipitate was in clean, sterile glass tubes. A sterile sugar cheddar solution was added in an amount equal to the precipitate. Then, the solution was shaken well until well mixed, and left for 5 minutes in a centrifuge at a speed of 4000 rpm. After removing the glass tubes, it was left for 15 minutes at room temperature, then withdrawn. The supernatant was obtained by using a Pasteur pipette and placed in a clean glass tube, and the flotation process was repeated three times where the supernatant was mixed together, then distilled water was added to the supernatant which was collected in a volumetric flask at a ratio of 1:10 respectively. diluted in centrifuge laboratory tubes and precipitated at 3000 rpm for 5 min, the precipitate was collected in clean, sterile tubes and examined under a microscope for presence of Oocysts.

2.2.5 Sterilization Oocysts

For the purpose of sterilizing the Oocysts, a solution of 6% sodium hypochlorite was added depending on the specific density (Density Gradient) to the sediment and they were mixed well using the Vortex vibrating mixer device, then the mixture was placed in a flask containing a magnetic electrode and its nozzle was covered with aluminum foil to prevent contamination, then the flask was placed on the motor device Magnetic Sterrier Duration 20-30 minutes (Davis et al., 1963) Pour the solution into test tubes with the addition of distilled water and carefully over the surface of the solution forming a separate layer, and after centrifuging at 1000 rpm for 10 minutes, a layer is formed. Separate between the water and the white sodium hypochlorite solution, which represents the sterile pure Oocyst (Jorgensen et al, 1997), as they were withdrawn using a Pasteur pipette and placed in other tubes and a washing process was performed to get rid of the sodium hypochlorite solution by adding distilled water and conducting a centrifugal process. For 7-8 times until the smell of the sodium hypochlorite solution disappears, the sediment was taken from each tube and collected in a clean and sterile glass container after examining it to ensure the presence of Oocysts (Ayaz et al., 2008).

2.2.6 Oocysts calculator

The sacs of mature (sporulated) oocysts were calculated in one milliliter, after taking 10 \(\mu\text{l}\) from the sample and diluted with 10 \(\mu\text{l}\) with distilled water, according to the number of sacs of mature eggs in the entire prepared slide, and since one milliliter is equal to 1000 \(\mu\text{l}\), the resulting number of oocyst was multiplied Mature x 100, and the percentage...
of sporulated oocyst was extracted by calculating 100 egg sacs in the prepared slide and using the following equation:

**Percentage of Oocysts % : The number of sporulated Oocysts \ number of Oocysts \times 100**

### 2.2.7 Enlargement and activation of Oocysts
The isolation was multiplied in the laboratory by infecting Ross 308 broiler chicks with multiple passes as follows:

After purification of the isolation, it was activated and the Oocysts were multiplied, as the study was conducted in a special laboratory inside the house from 1/4/2019 until 31/12/2020, 120 unsexed one-day-old (308 Ross) broiler chicks (males and females) were used. With an average weight of 380.5 g per chick, which was prepared by the Samarra hatchery, cages (100 cm length x 50 cm width x 60 cm height) were used to represent a repeater of the experiment and 4 chicks were placed inside each cage. With a depth of 3 cm.

The experimental pathological infection was caused for chicks of different ages (14, 21, 28, 35, 42, 49, 56, 63, 70, 77 days) and each experiment lasted 14 days after giving the parasite dose, and they were infected using a 5-ml Syringe plastic syringe. They were inserted orally directly into the intracrop with a suspension (3.06 ml) containing 50,000 sporulated egg sacs / chick, 3 replicates were used for one treatment (1998). With 5% potassium dichromate, the litter was collected weekly (twice for one experiment), the remaining chicks were dissected and the fatalities were also calculated.

### 2.2.8 Measuring the lowest dose of juvenile mature egg Oocysts , 50% mortality in the fertilized chicks
The method of researchers (1970) Morehouse and Baron was used in calculating the lowest dose of mature Oocysts to cause 50% mortality in the infected chicks as follows:

**A** - 120 Ross 308 chicks were randomly distributed to six treatments, each treatment had four replicates, and each replicate had five chicks (20 chicks/treatment).

**B** - Chicks were infected through the intracrop at the age of 30 days, according to the following dose: The first treatment: 50,000 sporulated Oocysts (3.06 ml). The second treatment is distilled water (control).

**C** - The fatalities were recorded from the beginning of the experiment until the end of the experiment.

### 2.3 Preparation of silver nanoparticles
Skyspring Silver nanoparticles were obtained in powder form by nanomaterials company, where the company stated that the percentage of purity of nanoparticles is 99.9%, which means that the product is of high purity and few impurities are negligible. It also mentioned that the secondary particle size is from 50-60 nanometers and the surface area 12 square meters per gram and the shape of the nanoparticles is spherical. Also, the company stated that the bulk density ratio is 0.35 grams per cubic centimeter and the true density is 10.5 grams per cubic centimeter. The company also mentioned the proportion of other elements or impurities in ppm: < 200 Cu < 100, Bi: 100, As: < 5, Al 100 > Si: < 50, Ni as in the picture (3-1).
III. RESULTS AND DISCUSSION

The Coccidioidomycosis E. tenella parasite was isolated and diagnosed from the cecum area of sick chickens brought to the veterinary hospital and veterinary clinics from poultry farms, as the number of examined received cases was 397, and after the examination using the direct wet swab first, it was noted that 221 cases of imported chickens were infected with coccidiosis and a total infection rate it reached 55% and the number of uninfected cases was 175 (Table 4-1).

Table (4-1): sample collection area number of positive and negative cases and percentage of total infection by direct swab wet.

<table>
<thead>
<tr>
<th>Sample collection area</th>
<th>The number of sample examined</th>
<th>The number of sample</th>
<th>Parasite detection method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Samarra</td>
<td>45</td>
<td>28</td>
<td>17</td>
</tr>
<tr>
<td>Dujali</td>
<td>70</td>
<td>43</td>
<td>27</td>
</tr>
<tr>
<td>Balad</td>
<td>54</td>
<td>24</td>
<td>30</td>
</tr>
<tr>
<td>Ishagi</td>
<td>97</td>
<td>63</td>
<td>34</td>
</tr>
<tr>
<td>Tikrit</td>
<td>35</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>Dhuluiya</td>
<td>38</td>
<td>15</td>
<td>23</td>
</tr>
<tr>
<td>Peggy</td>
<td>25</td>
<td>17</td>
<td>8</td>
</tr>
<tr>
<td>Sharaqat</td>
<td>33</td>
<td>14</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>397</td>
<td>221</td>
<td>175</td>
</tr>
</tbody>
</table>

**Chi-Square = 16.054  P-Value = 0.025***

Experimental Study

4.1: Clinical signs and symptoms

Through the daily follow-up of the chicks after being dosed with mature egg sacs and the experimental infection, it was noted that clear clinical signs appeared from the fourth day of infection, represented by lethargy and lack of feed consumption due to loss of appetite and dirty feathers, as well as coarseness and drooping of the wings with the pallor of the crest, and the chicks gathered in one place in search of warmth, picture (4-4).
On the fifth day, the signs and symptoms appearing on the infected chicks increased, as the chicks suddenly stopped eating feed and water and the appearance of bloody diarrhea, which increased in intensity with the continuation of the infection until it reached a state of acute bloody diarrhea. On the sixth and seventh days, an increase in the severity of the clinical signs visible on the chicks was observed, as lethargy and fatigue increased and some chicks became unable to stand and walk, an increase in the arching of the back, lowering the head from the level of the wing, closing the eyes, and complete abstinence of most affected chicks from eating feed and water for most of the affected chicks and joking Weakness and weight loss with the appearance of bloody diarrhea, picture (4-5).

The ingestion of the chicks of foreign rose hens with eggs of the eggs of the parasite coccidioidomycosis led to the occurrence of symptoms and signs of disease, it was noted through the results that the infection with the parasite *E. tenella* Severe clinical signs and symptoms were very clear in the chicks of foreign rose chickens, and this is consistent with what he mentioned Al-Alwani et al., (2008) It was found that the symptoms and signs of the disease were the general weakness of the bird, emaciation, extreme fatigue, a noticeable decline in the speed of growth, and the reluctance of some infected chicks to eat feed and water, as well as severe bloody diarrhea as a result of the penetration of spores into the epithelial tissue of the ceca, and the growth and development of the parasite to the stage of the Metaphase stage within the epithelial cells of the ceca, which leads To damage the capillary blood vessels and the occurrence of hemorrhage as shown by Al-Tai et al.,(2012) The increase in the clarity and severity of symptoms and clinical signs appearing on the chicks of foreign chickens may be caused by the high growth rate of the chicks of foreign chickens because they are meat chickens, causing them stress, and this is often done at the expense of their immunity and resistance to diseases, and the current study agreed with study Al-Alusi, (2014) when studying the effect of IgY immunoglobulin on experimentally infected chickens with cecal coccidioidomycosis *Eimeria tenella*.

The results also showed the emergence of a slight improvement and the disappearance and fading of some symptoms and clinical signs that appear gradually from some infected chicks that are not dead over time and their survival by overcoming the infection and this is consistent with what was reached Zulpo et al (2007), , that this improvement that occurred in some of the infected chicks may be due to the immunity formed by them over time, but despite this simple improvement and self-healing, they remained meager and their weights were low and below the required level and did not reach the normal condition infection , which negatively affected the digestion and absorption of nutrients.

4.2.2: Macroscopic pathological changes

The chicks that were dosed with sporulated and destroed egg sacs due to the parasite showed congestion in the internal organs, including the liver and kidneys, and the enlargement and expansion of the cecum and the thickening of its wall was noted, with the observation of hemorrhagic lesions, in addition to the presence of bloody stools in the contents of the cecum, When dissecting the dead chicks as a result of the infection weekly to observe the pathological effect of the parasite on the internal organs of the body, the chicks of the experimentally infected *E. tenella* species showed gross pathological lesions after the experimental infection was caused. Congestion was observed on the outer wall of the cecum Picture (4-6) and (4-7) after opening them with scissors, ulcers were observed with the cecum filled with blood and fluids mixed with stool, picture (4-8).

The most important macroscopic pathological changes observed on the parts of the alimentary canal in chicks infected with *E. tenella* are severe congestion on the outer wall of the cecum and the presence of ulcerative lesions of large size and fullness of the cecum. The presence of blood clots and an increase in size to weakness with the progression of infection and this is consistent with what reached by each all Haug et al.,(2008) and Al-Hamdani (2009). This may be due to the large numbers of different parasite stages present in the intestines of foreign chickens and causing them to penetrate and destroy the jejunum epithelium and to show macroscopic lesions. The macroscopic lesions were also observed on the third day during the first metaphase stage when a slight enlargement of the cecum appeared with the presence of necrotic areas of small size in the layer Epithelial lesion of the cecum. On the fourth day, cecal inflammation is observed in mild cases +1, In the most severe cases +2, the macroscopic lesions are represented by the presence of white and gray foci in the wall and lining of the cecum, in addition to the presence of simple bleeding diffuse on the wall of the cecum and a little blood with shedding inside the cavity, and in severe cases +3 inflammation is noted with the size of the cecum to three times the size Normal with the appearance of multiple irregularly shaped hemorrhagic spots on the serous surface with their cavity containing liquid and clotted blood, which shows the color of the cecum from the outside in a dark
brown color, and in very severe cases +4 on the sixth and seventh days the contents of the cecum harden and it is called the cecal core which consists of clotted blood and remnants of the epithelium and large numbers of egg sacs shed with guano, with a large enlargement in the size of the cecum (Imre, 2012). The study also agreed with the findings of Naim (2021) in his study, where he mentioned that the infection with the parasite was characterized by the occurrence of congestion of the cecum with blood and the mixing of blood with the remains of dead tissue and fluids.

Some chicks infected with coccidioidomycosis that did not die and with the passage of time begin to self-heal, although they remain lean, which is what he indicate Zulpo et al., (2007) this is due to the cases of shedding, growth and regeneration in the affected areas of the cecum.

4.2.3: The number of egg sacs thrown OPG

The results shown in Table (4-6) showed that there were significant differences at the level of (P≤0.05) between the groups for the number of egg sacs shed with litter faeces in the groups infected with the parasite E.tenella and the treatment by adding different treatments, where the highest value of the number of eggs laid in the litter was recorded after the introduction of the experimental infection was in the group that was treated by adding silver particles at 680 egg bags / gram at a concentration of 30 mg when compared with the infected control group, which recorded 60708 egg bags at the end of the experiment, table (4-6).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>The number of egg sacs at the end of the experiment</th>
<th>protection ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>silver particles</td>
<td>30 mg</td>
<td>680</td>
<td>37.942</td>
</tr>
<tr>
<td>Control</td>
<td>=</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>positive</td>
<td>=</td>
<td>60708</td>
<td>0</td>
</tr>
</tbody>
</table>

The results of the current study showed a clear discrepancy in the rate of the number of egg sacs thrown with the faeces in the litter. The appearance of the number of E. tenella egg sacs is large in the group of infected chicks compared to the rest of the groups, evidence of the severity of the infection. The differences in the number of egg sacs thrown are considered one of the strongest indicators that many researchers rely on for the purpose of assessing the severity of injuries and the treatments used to control coccidiosis, and this is consistent with what was stated Shareef (2010) it was noted that the highest rate of shed eggs, with a significant difference, was for the infected chicks, compared to what was shed by the chicks of other groups.

The low number of E.tenella egg sacs excreted with feces after administration of different treatments of plant extracts, silver nanoparticles and amprolium compared with the group of infected chicks confirms that the treatments used were effective and containing vitamins led to the stimulation of the intestine and the epithelial tissue lining the intestine and thus leads to the stimulation of proteins Immunodeficiency, which caused obstruction of parasite proliferation and reduction of egg sac numbers in the treated groups (Lee et al., 2011). And it agreed with the findings of Meskerem and Boonkaewwan, (2013) where it was observed that the number of shed eggs of Eimeria tenella was reduced in vaccinated groups by using egg sacs of different types of Eimeria compared with the control group, resistance and age of the infected chicks, and the current study agreed with a study Mashtat,(2013) where it was noted a decrease in the rate of egg sacs of the parasite excreted with feces when he studied the effect of vitamin A and vitamin E on live and heat weakened Eimeria infected chicks, and the current study agreed with the study of Al-Shaibani (2015) where he noticed a decrease in the rate of egg sacs of the parasite excreted with feces when he performed a diagnostic study Molecular, hematological and biochemical parameters of Eimeria spp. cocci in naturally and experimentally infected chickens in Al-Diwaniyah Governorate.

4.2.4: Blood picture tests

It was found that there was a significant increase in all blood tests for the negative control treatment compared to the positive control. It was also shown that the treatment of silver nanoparticles at a concentration of 30 mg in their hematological characteristics in terms of returning their proportions to the normal state compared with the positive control.
4.2.4.1: Packed cell volume (PCV)

The blood picture is one of the most important indicators of the pathological effects of infection, and the results of the current study showed that the parasite has an important effect on the volume of compressed blood cells in the infected chicks, causing a highly significant decrease in the values of hemoglobin concentration, the volume of compacted blood cells and the total number of red blood cells, as the results shown in Table (4-7) There were significant differences in the treatments at the level (P≤ 0.05) in the volume of pressurized blood cells. The highest values of the treated groups were recorded after induction of infection by the chicks that were treated with silver nanoparticles at a rate of 30.397% at a concentration of 30 mg Silver particles The lowest value recorded by the untreated infected group was 18.497% during the current study.

Table (4-7): Effect of silver nanoparticles on measurements of PCV, Hb, RBC, and WBC in chickens experimentally infected with E. tenella parasite.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>PCV %</th>
<th>HB dl/mg</th>
<th>RBC *10 ml/ Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>silver particles</td>
<td>30mg</td>
<td>23.280±0.807</td>
<td>13.037±0.287</td>
<td>3.250±0.0800</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>29.187±1.539</td>
<td>13.507±0.573</td>
<td>3.210±0.210</td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td>18.497±0.687</td>
<td>7.023±0.804</td>
<td>1.817±0.498</td>
</tr>
<tr>
<td>statistical analysis</td>
<td></td>
<td>F-Value=36.87**</td>
<td>P-value=0.0008</td>
<td>F-Value=7.24**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>F-Value=0.0003</td>
</tr>
</tbody>
</table>

The group treated with silver particles achieved the highest values compared to the control group, and the chicks of the untreated infected chickens in this study recorded a high decrease in the concentration of blood cell volume compared with the chicks of healthy chickens, which was attributed by Hirani et al., (2007) to the reason for this decrease in the values of the image criteria. In the blood, the rate of metabolic processes decreased as a result of infection with the parasite E. tenella, and this result is in line with what was found by Mashhat, (2013) he explained the reason for the phenomenon of low volume of pressurized blood cells in infected chicks due to several factors, including bleeding and the occurrence of parasite infection coincided with the secondary effects accompanying this infection in the intestines of lack of feed intake and lack of benefit from food as a result of changes in digestion and absorption of those materials and this is what This negatively affected the health of the infected chicks and the occurrence of iron deficiency and then anemia, as the poor health condition that was evident on the infected chicks, which led to a severe decrease in the concentration of hemoglobin in the blood, the volume of compacted blood cells and the total number of red blood cells, and the current study agreed. With the findings of Al-Shaibani (2015) when he conducted a molecular, hematological and biochemical diagnostic study of the types of cocci in naturally and experimentally infected chickens in Al-Diwaniyah Governorate.

4.2.4.2: Hemoglobin concentration Hb

The results shown in Table (4-7) showed that the group treated with silver particles achieved a significant superiority with a level (P≤ 0.05) in hemoglobin concentration over the course of the experiment, where the highest values were recorded at 15.570 mg/100 ml blood at a concentration of 30 mg and when The concentration of 30 mg of silver particles for the current study compared with the healthy control group, which recorded 13,507 mg/100 ml of blood in the current study. The lowest value recorded in the untreated infected group was 7.023 mg/100 ml blood at a concentration of 300 mg during the current study.

The group treated with silver particles achieved the highest estimates due to the therapeutic additions, followed by the control group because it was not infected, and the chicks of chickens infected with E. tenella parasite in this study recorded the lowest values in hemoglobin concentration, and this result is in line with what was found by Al-Shammari,(2010), and Merck,(2011) ) where it was shown that infection with the parasite E. tenella causes the disruption of the mucous and submucosal layer due to the attack of the second generation of phylloxera of the epithelial layer of the ceca, and thus a disruption in the work of the apocrine glands, which reduces the efficiency of absorption as well as the loss of large quantities of blood as a result of infection Meskerem et al., (2013) also indicated that infection with E. tenella will lead to severe anemia represented by bird weight imbalance and low RBC, PCV, and Hb values, and that recovery from infection will lead to the return of these percentages to their normal state.

4.2.4.3: Total red blood cell count RBC
The results shown in Table (4-7) showed that there were significant differences at the level of \( P \leq 0.05 \) in the total number of red blood cells among the chicks of groups that were treated after the introduction of infection with *E. tenella* parasite. The highest values of treatments were recorded by the group with silver particles. And particles of \( 3,467 \times 10^6 \) cells / mm\(^3\) blood at a concentration of 30 mg of silver particles compared with the negative control group, which was recorded at \( 3,467 \times 10^6 \) cells / mm\(^3\), while the lowest value was recorded by the affected group, which amounted to 1.817 mg / 100 ml of blood in the current study.

The group treated with silver particles recorded the highest values in the total number of red blood cells during the current study due to the therapeutic additives that were used, while the chicks of chickens infected with *E. tenella* parasite in this study recorded the lowest values in the total number of red blood cells compared with those of healthy chickens, which was attributed Hirani et al., (2007) caused the decrease in the total number of red blood cells to hemorrhage that occurred in the cecum, which led to the loss of quantities of blood and thus the decrease in the total number of red blood cells, in addition to obstructing the normal digestion and absorption processes, which led to a lack of primary nutrients that. The body needs it in the processes of growth, construction and renewal, and the current study agreed with the findings of Fahd et al., (2010) when studying the evaluation of the effectiveness of garlic extract and pomegranate peels on some blood values in chickens infected with the parasite Eimeria tenella, and the results also agreed with Al-Shaibani, (2015).) when he carried out a diagnostic molecular, hematological and biochemical study of the types of coccidioides in naturally and experimentally infected chickens in Al-Diwaniyah Governorate.

4.2.5: Biochemical tests for blood serum

It was found that there was a significant increase in all serological tests for the negative control treatment compared with the positive control. It was also found that the most significant treatments were the treatment of silver nanoparticles at a concentration of 30 mg of silver particles in their serum characteristics in terms of returning their ratios to the normal state compared to the positive control.

4.2.5.1: Glucose of serum

The results shown in Table (4-8) showed a decrease \( P \leq 0.05 \) in the blood glucose concentration of chickens infected with *E.tenella* parasite. The highest values were recorded among the groups treated with silver particles at 217.00 mg/100 ml blood serum at The concentration of 30 mg of silver particles compared to broilers of healthy control groups, which showed a significant increase in blood glucose concentration by 205.33 mg / 100 ml of blood serum, while the affected group recorded the lowest values in glucose concentration of 162.00 mg / 100 ml of blood serum during the current experiment.

Table (4-8): Effect of Silver Nanoparticles on Measurements of RBs, CHOLESTROL, TRIGLYCERIDE in Chicks Experimentally Infected with *E.tenella*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>RBs</th>
<th>CHOLESTROL dl/mg</th>
<th>TRIGLYCERIDE dl/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>silver particles</td>
<td>30mg</td>
<td>196.67±5.86</td>
<td>95.67 ±3.21</td>
<td>26.33±1.528</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>205.33±5.03</td>
<td>111.00±12.77</td>
<td>37.667±1.528</td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td>162.00±3.00</td>
<td>70.49±8.28</td>
<td>16.667±1.155</td>
</tr>
<tr>
<td>statistical analysis</td>
<td></td>
<td>F-Value=13.13*</td>
<td>F-Value=70.76**</td>
<td>F-Value=9.57**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P-value=0.0006</td>
<td>P-value=0.0005</td>
<td>P-value=0.0005</td>
</tr>
</tbody>
</table>

The group treated with silver nanoparticles recorded the highest values in glucose concentration due to the therapeutic additions of silver particles as a prevention of infection, which led to an improvement in the amount of feed consumed and thus led to an increase in glucose concentration, while the lowest values in the concentration of glucose were recorded in the chickens infected with the parasite *E. tenella* give any treatment, as poultry uses its blood glucose for many functions, including energy production, cellular oxidation, and building glycogen in the liver and muscles (Tokushima et al., 2003). These results are consistent with the findings of Al-Sultani, (2011) where he showed that a significant decrease in the glucose concentration of chicks infected with the parasite *E.tenella* may be due to the lack of resistance of the infected chickens to the rose, the size of damage, and the intestinal disturbance caused by infection, as well as the virulence and the high severity of the disease caused by infection. Characterized by the parasite, as well as between the Mshatat, (2013) the reason for the low percentage of glucose in the infected group is that severe infection with the parasite may cause a decrease in

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metabolic processes and inhibition of the process of converting glycogen to glucose in the liver due to failure in liver functions.

4.2.5.2: The concentration of cholesterol in the blood serum

Where the results shown in Table (4-9) showed that there were significant differences in the treatments at the level (0.05 ≤ P) in the concentration of cholesterol, the highest values were recorded for the group treated with silver nanoparticles at 122.67 mg / 100 ml of blood serum at a concentration of 30 mg of silver particles. During the current study, the lowest value recorded in the untreated infected group was 70.49 mg / 100 ml of serum during the current study, Appendix (4-7).

The group treated with silver particles recorded the highest values in cholesterol concentration due to the therapeutic additions represented by silver particles as a prevention of infection, as it agreed with Machado, (2002) who mentioned that measuring the concentration of cholesterol in the blood serum is widely used to study the effect of disease in the liver and its functions for its direct contribution to Construct and that most of the parasitic diseases that affect birds cause inflammation and damage to the liver and consequently a defect in the process of cholesterol building, as well as Dalloul et al., (2006) mentioned that the low level of cholesterol in the blood serum is usually associated with cases of bacterial septicemia accompanying infection with coccidiosis, and liver diseases. And that the decrease in the level of cholesterol and total fat in the blood serum occurs due to a decrease in absorption in the intestine caused by inflammation of the intestine or a decrease in the effectiveness of the liver in the synthesis of fats, and the level of cholesterol in the blood serum decreases when an increase in thyroid activity occurs. E. tenella caused a significant decrease in the concentration of cholesterol in the blood serum over the course of the current experiment compared with the chicks of the healthy control chickens, and the reason may be due to the direct effect this parasite affects the liver’s performance of its vital functions, which negatively affects the cholesterol building process in hepatocytes, and this is consistent with what was reached (Mondal et al., 2011). The high level of hypercholesterolemia in chickens is associated with starvation and hypothyroidism that occurs when infected with E. tenella, or it may be that the high cholesterol in the blood is in positive control due to tissue breakdown during infection, which releases stored fats into the bloodstream and thus leads to its rise, or the reason may be the lack of muscle building due to the lack of good use of food, which leads to negative responses in muscle building and its lack of growth, which leads to its gradual atrophy and destruction, and the release of fat quantities from the muscle into the blood, leading to high cholesterol (Meskerem et al., 2013).

4.2.5.3: The concentration of triglycerides in the blood serum

The results shown in Table (4-7) revealed that there were significant differences (P ≤ 0.05) in the level of triglycerides in the blood serum between the groups that were treated after the introduction of infection with the parasite E. tenella. The highest values in the level of triglycerides were recorded by the group. Treatment with silver particles was 40.667 mg / 100 ml of blood at a concentration of 30 mg of silver particles, while the lowest value was recorded by the untreated infected group, which showed a significant decrease in the level of triglycerides by 16.667 mg / 100 ml of blood serum during the current study, Appendix (4-8).

The group treated with silver wormwood particles recorded the highest values in the concentration of triglycerides due to the therapeutic additives represented by silver particles as a treatment from infection, while the chicks of chickens infected with the parasite E. tenella the lowest values in glucose concentration, being infected and not thirsting for any treatment, as the decrease in the level of triglyceride in plasma Infection with E. tenella may be due to the main cause, which is anorexia and malabsorption of nutrients in the body. It has been pointed out by Zheng et al., (2012) that the high rate of fat mobilization produced from the fat depot is due to the disappearance of most of the fat tissue and the excretion of its components in the blood when infected with E. tenella, and this in turn will reduce thymine due to the process of reduced fat formation from carbohydrates, as well as cause disturbance In the process of synthesis of vitamin B in severe infection of the cecal cells, which may hinder the process of lipogenesis synthesis from carbohydrates, in addition, glucogons inhibit the synthesis of fatty acids, which may be the reason behind the decrease in the level of triglycerides in the plasma, it was mentioned Meskerem et al., (2013) the efficacy or activity of non-pancreatic glucagons will be greatly increased in plasma in case of cecal coccidiosis, and the increase in serum cholesterol with subsequent decrease in plasma triglycerides can be attributed to the lower bile secretion of cholesterol in anorexia, which caused the increase in cholesterol In the blood despite the decrease in synthesis, the hormone insulin is secreted when the blood sugar increases, and this hormone, when released in the blood, will stimulate the fat cells to secrete the enzyme
Lipoprotein Lipase and urges them to consume glucose, which is a source of glycerol in the synthesis of triglycerides.

Table (4-2): Effect of silver nanoparticles on ALBUMIN and Calcium measurements in chicks of experimentally infected chickens with the parasite E.tenella

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>ALBUMIN dl/g</th>
<th>Calcium L/ mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>silver particles</td>
<td>30mg</td>
<td>19.367±0.603</td>
<td>1.5±0.5</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>12.433±0.987</td>
<td>1.9±0.2</td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td>10.767±0.709</td>
<td>0.9±0.05</td>
</tr>
<tr>
<td>statistical analysis</td>
<td></td>
<td>F-Value=9.29** F-Value=27.79**</td>
<td>P-value=0.0005 P-value=0.0008</td>
</tr>
</tbody>
</table>

4.2.5.4: Calcium concentration in the blood serum

Where the results shown in Table (4-9) showed that there were significant differences in the treatments at the level (P≤ 0.05) in the calcium concentration. 2.0 mg / 100 ml of blood serum at a concentration of 30 mg of silver particles, while the lowest value recorded by the uninfected group was 1.0 mg / 100 ml of blood serum at a concentration of 0.9 mg / 100 ml of blood serum during the current study, Appendix (4-10).

The results of the current experiment showed that the level of calcium in the blood serum was affected, as the group treated with silver particles recorded the highest values in calcium concentration due to the therapeutic additions represented by silver particles as a treatment against the parasite, while it showed a significant decrease in the chicks of chickens infected with E.tenella parasite, where the lowest values were recorded in the concentration Calcium compared with the chicks of the treated groups and the chicks of the healthy control chickens because they were not given silver particles and because of the parasite’s ferocity. The deficiency in calcium level can be explained by the fact that after a period of infection and the formation of the vesicle around the parasite inside the cells, the parasite becomes able to withdraw calcium from the cytoplasm of the host cells. To the inner perimeter of the surrounding vesicle, due to the fact that the intervascular network of the parasite vesicle has a high affinity for calcium (Coppens and Joine, 2001).

The process of occupying the host cells is also accompanied by the secretion of proteins by the parasite, which is accompanied by an increase in calcium inside the cell, as well as the process of the parasite’s exit from the destroyed cells induced by the low concentration of potassium ions outside the cell accompanied by a high concentration of calcium inside the cells. Calcium is also necessary for the gliding movement of the parasite (Pomel et al., 2008), showed that hypercalcemia occurs with an excess of vitamin D3 in the blood, and hypocalcemia is usually associated with a low blood albumin level, and calcium ions are necessary for blood clotting. (Muhammed et al., 2012).

4.2.5.5: Albumin concentration in serum

The results shown in Table (4-8) revealed that there were significant differences (P≤0.05) in the concentration of albumin in the blood serum between groups after the introduction of infection with E.tenella parasite. The highest values were recorded by the group that was treated with silver particles by 20.463. mg/100 ml of serum at a concentration of 30 mg of particles, while the lowest value of albumin concentration was recorded by the untreated infected group, which reached 9.333 mg/100 ml of serum in albumin concentration during the current study, Appendix (4-11).

The group treated with silver particles recorded the highest values in albumin concentration due to the therapeutic additions represented by silver particles as a treatment from infection, while the chicks of chickens infected with E. tenella compared with the chicks of treated group and healthy control chicks, where Ruff and Augustine, (1982) stated that the amount of albumin will decrease when infected with E. tenella parasite with an increase in the amount of Alpha 1, Beta, and gamma 1 globulins, while when infected with E. tenella. E. acervulina, all proteins are reduced and the lowest are alpha globulins. While infection with E. maxima will lead to a decrease in albumin and alpha 1 globulin only. It has been demonstrated by Natalija et al. (2007) The electrical migration of
serum electrophoresis showed that albumin is the largest protein part in the blood serum of normal birds, and that the proportions change according to the type of disease, and these results were very close to what was reached by Mushtat, (2013) when studying the effect of vitamin A and vitamin E on infected chicks. Live and thermally weakened Eimeria parasite.

Picture (4-4): The appearance of clinical signs on chicks infected with E.tenella parasite.

Picture (4-5) Bloody diarrhea in chicks of chickens experimentally infected with the parasite E.tenella.
Picture (4-6): Enlargement of the cecum with blood and an increase in their size in chicks infected with E.tenella parasite.

Picture (4-7): Enlargement of the cecum with blood and an increase in their size in chicks infected with E.tenella parasite.

Picture (4-8): Mixing of blood with feces in the caecum of chicks infected with E.tenella parasite.

REFERENCE


