Effects of Silver – Copper (Core-Shell) Nanoparticles as Antiparasitic Drug In Some Blood Parameters of Mice

Alaa Adnan Aljanabi¹, A. M. Al-mussawi², Abbas Matrood Bashi³

¹Lect. Department of Medical Laboratory Techniques, AlSafwa University College _ Iraq, ²Prof. Life Sciences, Karbala University _ College of Education for Pure Sciences _ Iraq, ³Prof. Department of Anesthesiology and Intensive Care, Altuff University College, Iraq

Abstract

The current study included the synthesis and diagnosis of the silver-copper nanoparticles Ag-Cu NPs in the form of (core-shell), with the commercial drug Albendazole Alb loaded on it Ag-Cu NPs /Alb. Then to know its effect on some hematological parameters of the orally dosed mice at three concentrations (400, 800 and 1200) mg/kg. The results showed a variation in the effect according to the concentrations used compared with the commercial drug treatment and the control treatment using only distilled water.

Key words: Ag-Cu NPs, Ag-Cu NPs /Alb, Core-Shell, Blood parameters.

Introduction

Echinococcus is one of the diseases of animal origin (Zoonosis), its adult stage resides in the gastrointestinal tract of the final host, while its larval stage resides in the intermediate host organs in the form of hydatid cysts, it is considered one of the common diseases with a great health impact in various regions of the world, cystic Echinococcosis cause by Echinococcus granulosus considered the most common but the least dangerous compared with the other species E. multilocularis, as well as the species, alveolar Echinococcosis and E. vogeli that cause polycystic Echinococcosis. (CFSPH, 2020). This disease is more prevalent in rural areas compared to cities, where the countryside has the highest prevalence rates for being the main a for sheep and it is the main intermediate host for the parasite, and developing countries have the highest rates of infection for this disease, as it is one of the main health obstacles in them (1,2).

The disease in Iraq, in particular, is one of the main health problems (CDC, 2012). The central and southern regions lead the number of infections, including the governorates of Basra, Dhi Qar and Muthanna (2,3). It is considered one of the health, social and economic problems due to the lack of an effective anti-drug As well as the absence of clinical symptoms on the patient until advanced stages are reached in the affected organs, especially the brain and heart, and thus controlling the disease becomes very difficult, whether surgical or therapeutic, and because of this, scientists have directed to use many methods to reduce the spread of the disease (4). Numerous studies have proven that nanoparticles NPs as Ag NPs, Cu NPs, chitosan NPs and other metal oxides have inhibitory and toxic effects for many parasites including Giardia, Leishmania, toxoplasma, Plasmodium and even some types of insect larvae (5). Ag NPs is the most effective and present in direct contact with the environment and has important properties and high effectiveness against various parasites, as is the case with bacteria and viruses. For this reason, many studies have recommended the use of NPs as antiparasites because they have high efficacy and less harm, so they can be used as a successful drug in order to resist parasites and limit the spread of diseases that cause them (5).

Due to the introduction of manufactured NPs in many medical applications such as carrying medicines, medical devices nanodrug, it is expected that their uses
will increase more widely (6). The safety of using these NPs has been tested through the mouth, inhalation, skin, intravenous injection and others, there are many studies aimed at finding out the toxicity of NPs including silver NPs (7,8). However, there are many studies that have shown that nanoparticles cause clotting and hemolysis (9). But these particles differed in several aspects, including the size and method of preparation, as well as the lack of studies in this aspect led to the difficulty of determining the toxicity of these molecules or not (10).

Therefore, the current research has tended to study the effect of a mixture of Ag-Cu NPs in the form (core-shell) and its effect in the blood of mice at concentrations similar to the drug dose of the commercial drug Alb with the use of two double doses.

**Material and Methods**

**Laboratory animals**

Swiss albino *Mus musculus* of Balb/c strain was used in our present study. Obtained from the Iraqi Center for Research on Cancer and Medical Genetics / Al-Mustansiriya University. It was transported directly to the animal house, the temperature was set 25 ± 5, ventilation and moderate lighting (12 hours of light and 12 hours of darkness) with the provision of water and the necessary food integrated from the material of the pallet as it is supplied with it twice a day and the floor is cleaned. About sawdust every week to keep the cages clean.

**Preparation of NPs**

The Ag-Cu NPs was prepared from 10 mL (0.1 N) ascorbic acid (vitamin C) with 2 mL (0.1 N) calcium chloride, which represented the core, and 1 ml (0.1 N) as shell. The three solutions reacted at room temperature with continuous shaking for one hour, until a gray precipitate was separated from the filtrate, then was washed with distilled water and spread until dryness, after which it was thoroughly ground and kept in glass tubes at room temperature until use (Nadagouda&Varma, 2007). The concentrations used in the study were prepared (400, 800 and 1200) mg / kg and were dosed orally for two consecutive days, then blood was drawn directly from the heart.

**Preparation of commercial drug**

The commercial drug Alb was used in the study, it was purchased from a commercial store, in the form of 200 mg tablets, the manufacturer is Julphar company in the U.A.E., the used dose was 400 mg / kg, which is the medically recognized drug dose for this drug, as used as a positive control transaction.

**Loading the commercial drug on NPs**

To load the commercial drug Alb onto the Ag-Cu NPs, 250 mg of the drug was taken, and it was added to 250 mg of Ag-Cu NPs, added to it one milliliter of distilled water, mixed well on the magnetic stirrer for 12 consecutive hours, after shaking it was left to precipitate and the filtrate was discarded. The sediment was taken to wash with distilled water, then it was dried at room temperature, and ground and kept in glass containers until use.

**Diagnosis of NPs**

To ensure that the prepared compound carries the nanoscale characteristics and is properly prepared, a group of methods have been relied on, including FTIR, AFM and XRD. The formation of the (core–shell) characteristic of the compound was also confirmed by SEM examination. The diagnosis was made at the Al-Fadhel Foundation / Babel, Al-Hilla branch / Iraq for methods FTIR, FMA, either XRD and SIM, which took place in Iran.

**Results**

**Effect of Ag-Cu NPs & Ag-Cu NPs/Alb in WBCs**

The results showed, as shown in figure (1) that there was an increase in the number of WBCs when treating with Alb With significant differences comparing with control, when treating with Ag-Cu NPs there are significant differences with 400 & 1200 mg/kg. The same in the case of treatment with Ag-Cu NPs / Alb with concentrations of 400 and 800 mg/kg. When comparing the nanoparticles with Albendazole treatment, we find the following, there was a significant difference when
the treatment with a concentration of 1200 mg/kg of Ag-Cu NPs, while with Ag-Cu NPs / Alb the significant difference was at the concentration 400 mg/kg only.

![Figure (1): Effect of Alb, Ag-Cu NPs & Ag-Cu NPs/Alb in WBCs](image1)

* Significant difference with control, ^ significant difference with Alb

**Effect of Effect of Ag-Cu NPs & Ag-Cu NPs / Alb in RBC**

Figure No. (2) shows that the RBC did not increase in number when comparing Alb with the control treatment, and when the treatment with Ag-Cu NPs, the significant differences appeared with concentrations 400 and 1200 mg/kg, while there was no significant difference between the concentration of 800 mg/kg and the control treatment. While the three concentrations of Ag-Cu NPs / Alb did not show any significant differences. Likewise, when compared with Alb, the significant differences appeared only with concentrations 400 and 1200 mg/kg of the Ag-Cu NPs, and they did not appear with the three concentrations of Ag-Cu NPs / Alb.

![Figure (2): Effect of Ag-Cu NPs & Ag-Cu NPs/Alb in RBC](image2)

* Significant difference with control, ^ significant difference with Alb
Effect of Ag-Cu NPs & Ag-Cu NPs / Alb in HGB (g/dL)

There was no significant difference between the control treatment and Alb for the HGB rate, as was the case with the concentration 800 mg/kg Ag-Cu NPs, while there were significant differences for the concentrations 400 and 1200 mg/kg compared to the control. The concentrations 400 and 800 mg/kg of Ag-Cu NPs / Alb did not differ from the control rate, while a significant difference appeared at the concentration of 1200 mg/kg. The results were similar when compared with Alb, where the significance was shown with concentrations 400 and 1200 mg/kg for the Ag-Cu NPs treatment, while its appearance was limited to the concentration of 1200 for the Ag-Cu NPs / Alb treatment as show in figure (3).

*Significant difference* with control, ^ significant difference with Alb

Effect of Ag-Cu NPs & Ag-Cu NPs / Alb in PLT

As shown in figure (4), there was no significant difference between the control treatment and Alb. The results showed the effect of Ag-Cu NPs on platelets PLT with clear significant differences when comparing with the control treatment. Significant differences appeared with concentrations 400 and 800 mg/kg, while the concentration of 1200 mg/kg did not differ from control and did not show significant differences. As for the Ag-Cu NPs / Alb, its three concentrations did not differ from the control treatment. When comparing the two compounds with the treatment of Alb, the results were similar, as the significant differences appeared with the two concentrations 400 and 800 mg/kg only for Ag-Cu NPs. As for Ag-Cu NPs / Alb, the three concentrations did not show any significant difference.
Effect of Ag-Cu NPs & Ag-Cu NPs/Alb in MCH

There were no significant differences for the treatment of Alb with the control treatment, and when comparing the three concentrations of Ag-Cu NPs with the control, they had no effect and no significant differences were found, while the concentrations 400 and 1200 mg/kg for Ag-Cu NPs/Alb showed a slight significant difference from the control. When compared with the treatment of Alb, the concentration of 400 mg/kg Ag-Cu NPs/Alb only showed a significant difference, and the remaining concentrations did not show any significant differences, as the Ag-Cu NPs and its three concentrations had no effect different from that of Alb, as shown in figure (5).
Discussion

The changes occurring in the RBC, as shown in the results in figure (1), may indicate the effect of NPs on the maturation of RBC during the process of manufacturing hemoglobin in the bone marrow\(^{(11)}\).

The results showed a decrease in the number of RBC with some concentrations used for the Ag-Cu NPs, while it increased with other concentrations. The results of the study are in agreement with Ghaehnavi, et al.\(^{(12)}\) that used Au-NPs at different concentrations, with some increasing the number of RBC while decreasing with the other, and the reason for this was indicated that the nanoparticles affected the hormone erythropoietin, which stimulated the formation of RBC from the bone marrow. The slight decrease in numbers may be due to inhibiting the action of the hormone erythropoietin, a glycoprotein that stimulates the production of RBC\(^{(13)}\). A decrease in the concentration of the hormone erythropoietin may be the cause of the normal anemia\(^{(14)}\). The natural cause of anemia is iron deficiency, but the term hypochromic anemia is a term given to all types of anemia\(^{(15)}\). It is known that anemia occurs as a result of a decrease in the number of RBC, this decrease may result from a lack of iron, cobalamin or folic acid or one of the chronic diseases that affect humans, and sometimes due to some toxic substances that affect the number of RBC produced from the bone marrow, and exposure to NPs such as Ag-NPs by mouth or intraperitoneal injection causes a harmful effect on RBC\(^{(16)}\). Ag-NPs affect the blood parameters, causing a decrease in the number of RBC, in addition to affecting the functions of the immune system, causing various disorders in it\(^{(17,18)}\). It is also evident that the toxic effect of silver nanoparticles is due to their direct interaction with RBC, causing oxidative stress, damage to membranes and then their dissolution\(^{(19,20)}\). In addition to injuring the membrane, Ag NPs affect lipid peroxidation and the production of antioxidant enzymes, smaller particles are more capable of dissolving blood and destroying the membrane\(^{(19)}\). There are many factors that affect the toxicity of a nanoparticle such as shape, size, aggregation of particles, surface shape, function and chemical composition, and there are many other effects whose mechanisms are still poorly understood\(^{(21)}\).

In a study conducted by Al-Baker et al. (2020), it was concluded in a study that he conducted on rats that oral exposure to AG-NPs leads to a decrease in HB, HCT and the number of RBC, MCHC, MCH, MCV. In conjunction with an increase in the PLT and WBC, it was concluded that these compounds are toxic to the blood and this is not in agreement with the results of the current study.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References


14. RAJENDRAN V, KRISHNASWAMY K. EFFECT OF SOLANUM VILLOSUM (MILL.) EXTRACT AND ITS SILVER NANOPARTICLES ON HEMATOPOIETIC SYSTEM OF DIETHYLNITROSAMINE-INDUCED HEPATOCELLULAR CARCINOMA IN RATS.


18. Abdelhalim MAK, Moussa SAA. The Dimensional Hematological Alterations Induced in Blood of Rats. vivo; 2012.

