Effect of Oral Administration of *Spirulina platensis* and Silver Nanoparticles on the Health Status of Blood Pictures and White Blood Cells in Healthy and Hyperlipidemia Animals

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**Abstract:** The study was conducted in the laboratories of the Department of Food Sciences - College of Agriculture and in the animal house of the College of Veterinary Medicine at Tikrit University for the period from 1/9/2021 until 29/11/2021 with the aim of identifying the therapeutic ability of *S. platensis* at a concentration of 150 mg/ml and AgNPs at a concentration 10 mg/ml on the health status of blood pictures and white blood cell in healthy and experimental hyperlipidemia. The results showed a significant increase in the values of Red Blood Cells (RBCs), Hemoglobin estimation (Hb) and the proportion of Hematocrit (Hct) for healthy groups given orally of AgNPs (T1) and Spirulina (T2) or of both (T3) and for groups treated by oral administration of AgNPs (T5) and Spirulina (T6) or both (T7) compared to the T4 infestation group whose values for the above criteria were 6.49 × 10⁶/mm³, 11.16 g/dl, 37.86 %, respectively. As for the values of MCH and MCHC, they decreased significantly in the healthy and treated groups compared to the infection group. Oral administration of AgNPs and Spirulina also caused a significant decrease in the healthy groups T1, T2, T3 and treated T5, T6, and T7 groups compared with the infected group in total and differential numbers of white blood cells.

**Keywords:** *Spirulina platensis*, AgNPs, Blood pictures, white blood cells.

**INTRODUCTION:** Atherosclerosis is a serious disease that affects humans as it is the primary responsible for heart disease, as it is described as a chronic arterial disease resulting from a dysfunction in the lining of blood vessels accompanied by thickening of the walls of the arteries and narrowing of their lumen (Xue, X. *et al.*, 2021). This disease occurs as a result of the deposition of fats, especially cholesterol, on the walls of the arteries, resulting in a loss of elasticity through a cumulative mechanism that may last between 20-30 years (Chen, J. *et al.*, 2021). Atherosclerosis cases were observed at ages that started from newborns and increased with advancing age, with high statistics indicating that cardiovascular diseases are the main cause of most deaths worldwide (Pesonen, E. *et al.*, 1999).

The consumption of food with a high content of fat and calories, in addition to the inflammation that affects the body’s tissues and organs, which gives the opportunity for the flow of cholesterol to the places of infection, is considered the main cause of atherosclerosis cases and the negative impact on the heart muscle, and thus the possibility of deaths (Lawson, J. S., & Glenn, W. K. 2021).

Although there are many drugs that have an anti-hyperlipidemia effect, they are not without negative side effects in the health of the body and its efficacy, so the research directions are to try to find the safest substances to get rid of high blood fats and the damage resulting from them to reach the maintenance of The health status of the human being and comes in the forefront of these materials are Spirulina algae and silver nanoparticles (AgNPs).
Spirulina is one of the most important Cyanobacteria species from the nutritional, medical and industrial point of view, as it contains many active elements and compounds that come in the forefront of protein at 70%, fats, carbohydrates, minerals, vitamins, sterols and other rare substances, making Spirulina the most important and prominent food in nature and it has been called Super Food (Suzery, M. et al., 2017; Lafarga, T. et al., 2020). Accordingly, Spirulina is considered a complementary treatment for many diseases, the most prominent of which is in reducing lipids and sugar in the blood and controlling the level of blood pressure (Finamore, A. et al., 2017) through its possession of many therapeutic mechanisms that include increasing the activity of antioxidant enzymes such as Glutathione peroxidase and Catalase. In addition to the effectiveness of its compounds in inhibiting the activity of the enzyme lipolytic lipase, which reduces the absorption of fats in the intestines (Hirahashi, T. et al., 2002).

Nanoscience or the science of fine particles with sizes between 1-100 nanometers is one of the most important modern technologies that are characterized by having unique properties different from the raw materials manufactured from them (Korkmaz, N. et al., 2020). One of the most prominent nanoparticles used globally is silver nanoparticles that have been used in various therapeutic aspects, most notably its high inhibitory ability against microbial cells, and the inhibition of cancer cells and in the treatment of many diseases, especially reducing blood fats by having a mechanism to inhibit the enzymes responsible for fat absorption in Intestines (Ninan, N. et al., 2020) has also been used in many commercial and medical products (Tortella, G. R. et al., 2020).

From the above and due to the high incidence of heart diseases and atherosclerosis in the entire community, the aim of the study was to try to clarify the effectiveness of Spirulina algae or AgNPs in improving the parameters of blood images and white blood cells related to the health of healthy male laboratory rats infected with healthy or experimental hyperlipidemia.

**MATERIALS AND METHODS:**

The study was conducted in the animal house - College of Veterinary Medicine - Tikrit University and used in the study 40 male white laboratory rats of age (8) weeks and weight (230-245g), divided into eight groups and placed in separate cages. High fat for four weeks, while the uninfected groups were fed a standard diet. Animals were given S. platensis at a concentration of 150mg/ml and AgNPs at a concentration of 10mg/ml.

The experimental animals were randomly distributed into eight groups, and each group had its own food, as follows:

- **The first group (control T0):**
  - The group of rats fed a standard diet.

- **The second group (T1 positive control):**
  - The group of rats fed the standard ration and the dose at a concentration of 10mg/ml of AgNPs.

- **The third group (T2 positive control):**
  - The group of rats fed the standard ration and dosed with a concentration of 150mg/ml of Spirulina.

- **Fourth group (T3 positive control):**
  - The group of rats fed the standard ration and dosed with a concentration of 10mg/ml of AgNPs + 150mg/ml of Spirulina.

- **The fifth group (T4 injury group):**
  - The group of rats fed a high-fat diet and hyperlipidemia, which were left untreated.

- **The sixth group (T5 treatment):**
  - The group of hyperlipidemic rats dosed with a concentration of 10mg/ml of AgNPs.

- **Group VII (T6 treatment):**
  - The group of hyperlipidemic rats dosed with 150mg/ml of Spirulina.

- **The eighth group (T7 treatment):**
  - The group of hyperlipidemic rats dosed with a concentration of 10mg/ml AgNPs + 150mg/ml Spirulina.

- **Measured Standards:**
  - After the end of the study period, blood was drawn from the animals and placed in tubes containing anticoagulant (EDTA) to measure blood pictures and white blood cells. Complete blood pictures (CBC) were performed with a French-made Hematology analyzer. It is a multi-scale automated system analyzer designed for diagnostic tests in clinical laboratories, 0.5ml of blood was taken and placed in the tubes for analysis of the whole blood pictures containing (EDTA) to prevent blood clots the tube was shaken well (slowly) after which it was placed in the place designated for it in the device for the purpose of completing the analysis process. The blood picture criteria included the white blood cells (WBCs), red blood cells (RBCs), hemoglobin Hb, monocytes (Mon%), lymphocytes (Lym%), Gra%, platelet count PLT, Hematocrit (Hct), average cell size (MCV) erythrocyte hemoglobin level (MCH), mean hemoglobin concentration (MCHC).

**RESULTS AND DISCUSSION:**

- **Effect of Oral Administration of Spirulina Algae Powder and AgNPs on Blood Picture Parameters of Rats:**
  - The blood picture parameters were estimated, which included the number of RBCs, hemoglobin concentration (Hb), hematocrit (Hct), erythrocyte hemoglobin (MCH), and erythrocyte hemoglobin...
concentration (MCHC), which are important criteria for assessing the health of animals. The deviation of these values from the normal limit is often associated with pathological or environmental stress. Table 2 shows the results of the effect of oral administration of Spirulina powder and silver nanoparticles on blood picture parameters of healthy and Hyperlipidemia animals. The results showed a significant decrease (P<0.05) in the total numbers of RBCs in the injury group (T4) and it was at 6.49 × 10^6/mm^3 compared with their numbers for the control group (T0) and the positive control groups T1, T2, T3 and the treatment groups T5, T6, T7, in which there was a positive improvement in the values of the measured parameters significantly, and its maximum significant increase was in the positive control group of healthy animals dosed with Spirulina (T2), and its value was at (8.51) ×10^6/mm^3.

As for the level of hemoglobin Hb, the oral administration of Spirulina powder and silver nanoparticles improved the health status of healthy and hyperlipidemia animals, where the results converged with the control group, and the highest significant increase (P<0.05) occurred in the positive control groups, specifically in the group dosed with Spirulina (T2) and the group synergistically dosed with Spirulina and AgNPs particles (T3) whose values were at 12.86 and 12.91g/dl, respectively, while significantly decreased in the infected group (T4) compared with the control group, and the highest significant increase was in the positive control group (T0), which was at 16,16 pg and 28,56g/dl, respectively, and the case of hyperlipidemia caused an increase in stress on the cells of animals and an increase in their values significantly, where it was at 19.73 pg and 32,16g/dl, respectively. The case of oral administration Rats with hyperlipidemia of either AgNPs (T5) or Spirulina (T6) or both (T7) caused a sensitive modification of both MCH and MCHC values and were in the case of the T5 group at 16.16 pg and 29.87g/dl respectively and in the case of Spirulina administration (T6) at 16,83 pg and 29.66g/dl, respectively, and in the case of T7, at 16.5 pg and 29.6g/dl, respectively.

It was clear from the results of table 1 that there was an improvement in the health status of rats given orally through an improvement in the values of blood picture parameters in all groups, which reflects the positive effect of silver nanoparticles and Spirulina powder on those parameters and consequently on the health of the animals.

### Table 1: Effect of oral administration of Spirulina powder and silver nanoparticles on blood picture parameters of healthy and hyperlipidemia animals

<table>
<thead>
<tr>
<th>Transactions</th>
<th>RBCs (×10^6/mm^3)</th>
<th>Hb g/dl</th>
<th>Hct %</th>
<th>MCH pg</th>
<th>MCHC g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>7.72b ±0.07</td>
<td>12.46b±0.09</td>
<td>43.63b ±0.12</td>
<td>16.16b ±0.56</td>
<td>28.56c</td>
</tr>
<tr>
<td>T1</td>
<td>7.58b ±0.45</td>
<td>12.40b±0.50</td>
<td>43.13b ±0.34</td>
<td>16.40b ±0.34</td>
<td>28.70c</td>
</tr>
<tr>
<td>T2</td>
<td>8.51a ±0.34</td>
<td>12.86a±0.34</td>
<td>44.80a ±0.34</td>
<td>15.90b ±0.12</td>
<td>28.36c</td>
</tr>
<tr>
<td>T3</td>
<td>7.93b ±0.43</td>
<td>12.91a±0.68</td>
<td>44.76a ±2.29</td>
<td>16.36b ±0.14</td>
<td>29.43b</td>
</tr>
<tr>
<td>T4</td>
<td>6.49c ±0.24</td>
<td>11.16d±0.57</td>
<td>37.86d ±1.77</td>
<td>19.73a ±0.17</td>
<td>32.16a</td>
</tr>
<tr>
<td>T5</td>
<td>7.50b ±0.55</td>
<td>11.87c±0.40</td>
<td>42.02c ±1.35</td>
<td>16.60b ±0.38</td>
<td>29.87b</td>
</tr>
<tr>
<td>T6</td>
<td>7.70b ±0.03</td>
<td>12.10c±0.52</td>
<td>44.10a ±1.45</td>
<td>16.83b ±0.76</td>
<td>29.66b</td>
</tr>
<tr>
<td>T7</td>
<td>7.83b ±0.22</td>
<td>12.50b±0.41</td>
<td>45.23a ±0.36</td>
<td>16.5b ±0.36</td>
<td>29.60b</td>
</tr>
</tbody>
</table>

Different letters in the same column indicate significant differences at the probability level of 0.05 ± = standard error. Averages are for five animals.
T0: healthy control, T1: positive control given AgNPs, T2: positive control given Spirulina, T3: positive control given AgNPs with Spirulina, T4: hyperlipidemia group without treatment, T5: treatment group given AgNPs, T6: treatment group given Spirulina, T7: treated group given AgNPs with Spirulina.

The results agreed with Abdel-Daim (Abdel-Daim, M. M. 2014), who explained the health benefits of Spirulina and its importance in ridding the body of the side effects of some drugs. An increase in RBCs and Hb values was noted, which reflects the improvement in the health status of the organism. The results also agreed with Bashir et al., (Bashir, S. et al., 2019) who noted an improvement in the health status of rats that were fed Spirulina compared to animals that were not given Spirulina through an increase in the values of some blood forms that included RBCs, Hb, and Hct. This gives an impression of the importance of Spirulina in treating blood diseases and the necessity of using it in the treatment of malnutrition.

The results also agreed with Nayyef and Thalij (Nayyef, S. H., & Thalij, K. M. 2020) who found a role of Spirulina at a concentration of 25mg/kg in improving the number of red blood cells and hemoglobin in rats infected with Pseudomonas aeruginosa.

The results also agreed with Raheem (Raheem, H. Q. 2018) who, in a study he conducted on rabbits that included intramuscular injection of AgNPs at a concentration of 50μg/kg, as he noticed an increase in the value of RBCs and Hct and a decrease in the value of MCH whose values were at 7.4 x 10⁶/mm³, 70, 6%, 17.6 pg, respectively, compared to its value in the control group for the same study, which was at 5.3 x 10⁶/mm³, 27.3%, 33 pg, respectively, and the results differed with it in the value of MCHC, which increased compared to its value in the group control and with Hb that decreased compared to its value in the control group.

The results converged with Lee et al., (Lee, J. H. et al., 2018) who found that the values of blood images for the same study (RBCs, Hb, Hct, MCH, MCHC) did not differ significantly in rats dosed with silver nanoparticles from the control sample.

**Effect of Oral Administration of Spirulina and Silver Nanoparticles on the Total and Differential Numbers of White Blood Cells:**

The effect of oral administration of Spirulina or silver nanoparticles alone or in combination to groups of healthy and hyperlipidemic rats on the total and differential number of white blood cells is shown in table 2.

The results showed that oral administration to healthy animals of AgNPs (T1) and Spirulina (T2) separately or together (T3) did not have a significant (P<0.05) effect on the total numbers of WBCs except in the case of administration together (T3) which Significantly reduced its numbers and it was at 6.66 x10⁷/mm³ compared with the control group (T0), whose value was at 7.30 x 10⁷/mm³, the case of oral administration to animals suffering from hyperlipidemia of either AgNPs (T5) or Spirulina (T6 ) or normal (T7) caused a significant improvement in the total numbers of white blood cells, which were at 7.67, 7.53, 7.70 x10⁷/mm³, respectively, compared with their total numbers in the infection group, which were at 8.64 x10⁷/mm³.

As for the effect of oral administration on the percentage of lymphocytes (Lym), the results indicated that there was no significant difference (P>0.05) in all positive control groups (T1, T2, T3) and treatment groups (T5, T6, T7) compared with the percentage of its presence in the positive control group (T5, T6, T7). The control (T0), whose percentage was at 71.93%, and thus, all groups decreased significantly compared to their value in the hyperlipidemia group (T4) whose lymphocyte values were at 79.83%, and this reflects the positive effect of Spirulina and AgNPs particles on Health and immune status of rat blood.

As for the Monocytes (Mon), it did not indicate a significant difference between the different groups, and the percentage of granulocytes (Gra) in the T4 group decreased significantly and was at 12,66%, while it usually increased significantly in the rest of the groups in the healthy animals (T1, T2, T3) as well as The treated groups of infected animals (T5, T6, T7) where there was no significant difference between them and the control group (T0), whose percentage was at 18.56%.

The results agreed with what was mentioned by Bashir et al., (Bashir, S. et al., 2019) who gave Spirulina to rats as a diet in order to observe its effect on them, as it was reported that there were no significant differences between the control group and the rest of the groups given Spirulina regarding the number of white blood cells (WBCs), and the same was true for Lym and Mon. The results are also in agreement with Hassan et al., (Hassan, N. S. et al., 2019) who found an effective role of Spirulina in eliminating the toxic effect of lead which reduced the value of WBCs, Lym and Mon compared to their value in the affected animal group.

The results agreed with Raheem (Raheem, H. Q. 2018) who observed that the values of blood images for the same study he conducted on rabbits that included intramuscular injection of AgNPs at a concentration of 50μg/kg, as he noticed an increase in the value of RBCs and Hb, and Hct. This gives an impression of the importance of Spirulina in treating blood diseases and the necessity of using it in the treatment of malnutrition.

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### Table 2: The effect of oral administration of AgNPs and Spirulina on the total and differential number of white blood cells

<table>
<thead>
<tr>
<th>Transactions</th>
<th>WBCs ×10^3/mm³</th>
<th>Lym %</th>
<th>Mon %</th>
<th>Gra %</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>7.30b ±0.38</td>
<td>71.93b ±2.89</td>
<td>4.50a ±1.34</td>
<td>18.56a ±1.69</td>
</tr>
<tr>
<td>T1</td>
<td>6.93bc ±0.77</td>
<td>73.53b ±2.99</td>
<td>4.70a ±0.26</td>
<td>17.76a ±0.72</td>
</tr>
<tr>
<td>T2</td>
<td>7.73b ±0.41</td>
<td>72.40b ±3.20</td>
<td>4.03a ±0.66</td>
<td>18.66a ±2.62</td>
</tr>
<tr>
<td>T3</td>
<td>6.66c ±0.61</td>
<td>73.40b ±2.96</td>
<td>4.13a ±0.39</td>
<td>17.46a ±1.88</td>
</tr>
<tr>
<td>T4</td>
<td>8.64a ±0.69</td>
<td>79.83a ±1.70</td>
<td>3.92a ±0.72</td>
<td>12.66b ±1.41</td>
</tr>
<tr>
<td>T5</td>
<td>7.67b ±0.45</td>
<td>72.22b ±3.64</td>
<td>3.80a ±1.07</td>
<td>18.97a ±2.64</td>
</tr>
<tr>
<td>T6</td>
<td>7.53b ±0.89</td>
<td>73.70b ±2.45</td>
<td>4.70a ±1.05</td>
<td>18.60a ±1.59</td>
</tr>
<tr>
<td>T7</td>
<td>7.70b ±0.98</td>
<td>74.03b ±1.47</td>
<td>4.03a ±1.08</td>
<td>17.23a ±0.38</td>
</tr>
</tbody>
</table>

Different letters in the same column indicate significant differences at the probability level of 0.05 ± = standard error. Averages are for five animals.

T0: healthy control, T1: positive control given AgNPs, T2: positive control given Spirulina, T3: positive control given AgNPs with Spirulina, T4: hyperlipidemia group without treatment, T5: treatment group given AgNPs, T6: treatment group given Spirulina, T7: treated group given AgNPs with Spirulina.

The results converged with what Lee et al., (Lee, J. H. et al., 2018) found in that the number of white blood cells and the percentage of lymphocytes and monocytes did not differ significantly in laboratory animals injected AgNPs at a concentration of 10 and 100μg compared with their numbers in the control group where there was a decrease but not significant in the dosed groups Compared with prepared in the control group.

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**Effect of Oral Administration of AgNPs and Spirulina on Platelets:**

Platelets (PLT) are one of the main components of blood, and their function lies in the formation of clots to prevent bleeding in the blood vessels, and the decrease or increase in the level of these plates causes thrombocytopeny, so it is an indicator of the health status of the organism.

The results in Figure 1 showed that the number of platelets in the blood of healthy rats given particles of AgNPs (T1) or Spirulina (T2) or together (T3) had a significant decrease (P<0.05) in their numbers and it was at 374, 376, 373 10⁹/L, respectively compared with their numbers in the control group (T0) which was at 383 10⁹/L that the condition of hyperlipidemia in rats caused an increase in the number of platelets in rats and it was at 495 10⁹/L that the condition of oral administration of each of AgNPs (T5) and Spirulina (T6) or together (T7) for animals suffering from hyperlipidemia, which caused a significant decrease in the numbers of those platelets and were at 414, 408 and 396 10⁹/L, respectively.
The different letters between the columns indicate a significant difference at the 0.05 probability level.

T0: healthy control, T1: positive control given AgNPs, T2: positive control given Spirulina, T3: positive control given AgNPs with Spirulina, T4: hyperlipidemia group without treatment, T5: treatment group given AgNPs, T6: treatment group given Spirulina, T7: treated group given AgNPs with Spirulina.

**Figure 1:** Effect of oral administration of Spirulina and AgNPs on platelet count (10⁹/L) in healthy and hyperlipidemic groups.

The results are in agreement with Hassan *et al.*, (Hassan, N. S. *et al.*, 2019) who found a role for Spirulina in decreasing platelet value in animals suffering from lead poisoning, as the 60-day dosing caused the PLT values in the treated group to be at 515 10⁹/L in male rats, while female rats Rats and for the same study, its values were at 445, 376 10⁹/L for each of the lead-infected group and the treated group, respectively.

The results converged with the study of Lee *et al.*, (Lee, J. H. *et al.*, 2018) who noticed a decrease in the platelet values in groups dosed with AgNPs particles at a concentration of 10 and 100µg per day compared to the control group, as the values were at 1176, 1123, 1053 K/µL for both the control group and the group. The results were in agreement with the study of Krutyakov *et al.*, (Krutyakov, Y. A. *et al.*, 2021) who noted that there were no significant differences between the group dosed with silver nanoparticles at a concentration of 2.25mg and the control group whose values were at 734, 732 10⁹/L.

**REFERENCES:**


