Effect of Oral Administration of *Spirulina platensis* and Silver Nanoparticles on the Health Status of Blood Lipids and Liver Enzymes 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 healthy blood lipids and liver enzymes in healthy and hyperlipidemia rats. The results of the biological experiment showed that hyperlipidemia had a negative effect on most of the animals' biological parameters, and that treatment with Spirulina and Nano silver alone or a combination of them had caused an improvement in the animals' biological parameters. The results showed that the oral administration of AgNPs (T1) and Spirulina (T2) and their mixture (T3) to healthy animals significantly (p<0.05) lowered the value of triglycerides to become at 106, 104, and 98 mg/dl, respectively, compared with their values in the control group (T0) of 151 mg/dl and hyperlipidemia (T4) caused an increase in its value to reach 357 mg/dl, and treatment with AgNPs (T5) and Spirulina (T6) or both (T7) caused a decrease in its values to become at 188, 173, 165 mg/dl respectively. As for its effect on cholesterol values, it caused a significant decrease in its values in groups T1, T2, and T3, which were at 95, 91, and 90 mg/dl, respectively, compared to its value in the animals of the T0 control group, which amounted to 97 mg/dl. As for its value in the T4 group, it reached 243 mg/dl, and in the treatment groups T5, T6, and T7 it reached 143, 123 and 117 mg/dl, respectively. Oral administration also caused a decrease in LDL and vLDL values and an increase in HDL values compared to the values in healthy control groups T0 and infected with T4. Oral administration of AgNPs and Spirulina also caused a significant decrease in healthy groups T1, T2, T3 and treated T5, T6, and T7 groups compared with the infected group in liver enzymes AST, ALT and ALK.

**Keywords:** *Spirulina platensis*, AgNPs, Blood lipids, liver enzymes.

**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, H. *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-hyperlipidemic 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 foregoing 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 or AgNPs in improving the vital parameters of blood lipids and liver enzymes related to the health of healthy male laboratory rats with 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 is drawn from the animals for the purpose of conducting the following biochemical tests:

- **Determination of Total Cholesterol Concentration in Blood Serum:**
  - **Basic principle:**
    - The cholesterol oxidase enzyme works on the oxidation of cholesterol in the presence of the enzyme peroxidase and in the presence of a hydrogen donor. The colorless substrate is oxidized to the pink dye quinonemine, according to the following equations:
Cholesterol esterase
Cholesterol esters $\rightarrow$ Cholesterol + free fatty acid

Cholesterol Oxidase
Cholesterol + O$_2$ $\rightarrow$ 4 cholestenona +H$_2$O$_2$

Peroxidase
2H$_2$O$_2$+phenol+4-Amino-antipyrin $\rightarrow$ Quinoneimine(pink)+4H$_2$O

The intensity of the carmine-red color of the resulting complex was measured using a spectrophotometer at a wavelength of 500 nm. The total cholesterol concentration is calculated according to the following equation:

$$\text{(A) Sample Cholesterol concentration} = \frac{\text{(A) Standard}}{\text{Standard Concentration}} \times \text{Standard Conc.}$$

(A) = Absorbance
Standard Concentration = 200 mg/dl

**Determination of Triglycerides Concentration in blood serum:**

The estimation of the level of triglycerides in the blood serum is carried out using a ready-made test kit (kit) manufactured by the French company Biolabo, based on the method (Fossati, P., & Prencipe, L. 1982).

**Basic Principle:**

This method is based on enzymatic analysis of triglycerides into free fatty acids and glycerol, which passes through a series of reactions to produce a pink complex according to the following equations:

Lipoprotein lipase
Triglycerides $\rightarrow$ Glycerol + Free fatty acids

Glycerol + ATP $\rightarrow$ Glycerol Kinase $\rightarrow$ Glycerol -3- phosphate + ADP

Glycerol -3-phosphate + O$_2$ $\rightarrow$ GPO $\rightarrow$ Dihydroxyacetone Phosphate +H$_2$O$_2$

H$_2$O$_2$+4-ChloroPhenol+4-amino-antipyrine $\rightarrow$ Quinoneimine (pink) + H$_2$O (coloured complex)

The color intensity of the complex resulting from the reaction is read by a spectrophotometer at a wavelength of 500 nm and the concentration of triglycerides is calculated as shown in the following equation:

$$\text{(A) Sample Triglycerides concentration (mg/dl)} = \frac{\text{(A) Standard}}{\text{Standard}} \times \text{Standard Conc.}$$

As (A) Absorbance.
Standard concentration = 200mg/dl.

**Determination of High Density Lipoprotein-cholesterol Concentration (HDL-C) in Blood Serum:**

**Basic Principle:**

The concentration of HDL-C in serum was estimated using a ready-made analysis kit from the French company Biolabo based on the enzymatic method that includes precipitation of LDL-C, VLDL-C and chylomicrons by adding phosphotinextic acid in the presence of magnesium ions, as the filtrate obtained after the separation process using centrifugation contains only HDL-C, to be estimated using the enzyme solution of cholesterol (Tietz, N. W., & Shuey, D. F. 1993).

**Procedure:**

A test tube was prepared and 50μl of blood serum and 50μl of precipitate were added, then mixed well and left at laboratory temperature for 10 minutes. Then the mixture was placed in a centrifuge for 15 minutes at a speed of 4000 rpm and the supernatant was separated from the precipitate. Three test tubes were taken and
RESULTS AND DISCUSSION:

- **Effect on Blood Lipid Profile Parameters:**

Determining the parameters of the blood lipid profile is of great importance in identifying the health status of the organism, as it clearly indicates the metabolic state between the components of fat and nutrients and their impact on the health of the body. Table 1 shows the effect of oral dosing of AgNPs and Spirulina on the blood lipid profile of hyperlipidemia animals. The results showed that the case of oral administration of both AgNPs and Spirulina caused a significant decrease in the level of triglycerides (TG), cholesterol (TC), and LDL cholesterol compared to their value in the infection group. The highest significant value (P<0.05) recorded for both TG and TC and LDL was in the group of infected animals fed a high-fat diet, which amounted to 357, 243, and 145mg/dl, respectively. Determining the parameters of the blood lipid profile is of great importance in identifying the health status of the organism because it clearly indicates the metabolic state between the components of fat and nutrients and their impact on the health of the body. Table (4-5) shows the effect of oral dosing of AgNPs and Spirulina on the blood lipid profile of hyperlipidemia animals. The results showed that the case of oral administration of both AgNPs and Spirulina caused a significant decrease in the level of triglycerides (TG), cholesterol TC, and LDL cholesterol compared to their value in the infection group. The highest significant value (P<0.05) recorded for both TG and TC and LDL was in the group of infected animals fed a high-fat diet, which reached 357, 243, and 145 mg/dl, respectively. The effect of oral administration on the parameters of TG, TC and LDL in healthy animals had caused the significant decrease in the above criteria, and their values in the case of oral administration of AgNPs (T1) were at 106, 95, 35.2 mg/dl, respectively, and in the case of administration Oral Spirulina (T2) had its value at 104, 91, 26.9mg/dl, and when given both (T3), its values were at 98, 90, 9.1 mg/dl, respectively, compared with its value in the control group (T0), which were at 151, 97, 34.5mg/dl, respectively, and the same situation was in the case of Very Low Density Lipoprotein (vLDL) compared to the control group.

In the case of animals suffering from experimental hyperlipidemia, these values started with a significant decrease and approached the normal level when dosed with AgNPs (T5) particles and were at 188, 143, and 69.3mg/dl, respectively, and in the case of oral administration of Spirulina (T6) it became at 173, 123, 47.4mg/dl, respectively, and when administered orally of their mixture (T7), their values were at 165, 117, 31.4 mg/dl, respectively, compared with their standards in the injury group (T4), which were at 357, 243, 145mg/dl, respectively.

Table 1 shows the values of High Density Lipoprotein (HDL) in which there was a significant increase (P<0.05) in groups of T1, T2 and T3 animals, which were at 38, 43.3, and 61.3mg/dl, respectively, compared to its value in the animals of the healthy control group T0, which was at 32.3mg/dl, the case of hyperlipidemia (T4) caused a significant decrease in its value to be at 26.6mg/dl, and the case of oral administration to rats Infected with hyperlipidemia with AgNPs and Spirulina and their mixture together caused
a modification of its value to be at 36.1, 41.5, and 26.6 mg/dl, respectively, compared to its value in the infestation group, which was at 52.6 mg/dl.

<table>
<thead>
<tr>
<th>Transactions</th>
<th>TG mg/dl</th>
<th>TC mg/dl</th>
<th>LDL mg/dl</th>
<th>HDL mg/dl</th>
<th>vLDL mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>±5.01</td>
<td>±8.02</td>
<td>±1.6</td>
<td>±3.84</td>
<td>±3.0</td>
</tr>
<tr>
<td>T1</td>
<td>±4.16</td>
<td>±9.02</td>
<td>±1.8</td>
<td>±2.76</td>
<td>±0.83</td>
</tr>
<tr>
<td>T2</td>
<td>±9.87</td>
<td>±6.55</td>
<td>±1.1</td>
<td>±2.38</td>
<td>±1.97</td>
</tr>
<tr>
<td>T3</td>
<td>±3.02</td>
<td>±6.53</td>
<td>±1.2</td>
<td>±7.88</td>
<td>±1.4</td>
</tr>
<tr>
<td>T4</td>
<td>±8.03</td>
<td>±6.35</td>
<td>±7.7</td>
<td>±4.25</td>
<td>±2.1</td>
</tr>
<tr>
<td>T5</td>
<td>±8.16</td>
<td>±3.49</td>
<td>±7.29</td>
<td>±5.21</td>
<td>±1.63</td>
</tr>
<tr>
<td>T6</td>
<td>±9.64</td>
<td>±8.55</td>
<td>±2.9</td>
<td>±5.71</td>
<td>±1.92</td>
</tr>
<tr>
<td>T7</td>
<td>±4.87</td>
<td>±3.26</td>
<td>±1.7</td>
<td>±3.86</td>
<td>±1.37</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.

**Table 1: Effect of Oral administration of Spirulina and AgNPs on blood lipid profile (mg/dl)**

The results agreed with Li et al., (Li, T. T. et al., 2019) who found that the use of Spirulina alga in the treatment of hyperlipidemic rats led to a positive modification of the values of TG, TC, LDL and vLDL in its low, as well as in modifying the value of HDL in its height, as Li et al., (Li, T. T. et al., 2021) mentioned the role. It is important for Spirulina to improve the health status of blood lipids and make it within the normal limits in mice fed foods with a high content of fats and carbohydrates, as the results agreed with Vide et al., (Vidé, J. et al., 2015). High fat diet.

The results also agreed with Kaur and Singhal (Kaur, S., & Singhal, B. 2012) who indicated lower values of TC, TG, LDL and vLDL in rats given AgNPs particles compared to the control group fed a high-fat diet, and agreed with Al-Dujaili and Al-Shemeri (Al-Dujaili, A. N. G., & Al-shemeri, M. K. 2016). Who found a role for silver nanoparticles in improving the health status of the blood lipid profile through a study conducted on rats fed a high-fat diet. The reason for the ability of nanoparticles to reduce blood lipids, especially cholesterol, could be due to their ability to inhibit the mechanisms responsible for cholesterol absorption in the body (Ninan, N. et al., 2020).

**Effect of Oral Administration of Spirulina and AgNPs on Liver Enzyme Parameters of Rats:**

The liver is one of the important organs of the body, as it produces many enzymes that have a vital role in catalyzing and regulating many chemical reactions, including the enzyme aspartate aminotransferase (AST), the enzyme that transports alanine aminotransferase (ALT), and the alkaline phosphatase (ALK), which are estimated in animals orally administrated groups of Spirulina and silver nanoparticles. The results in Table 2 showed that there was no significant difference (P>0.05) in the value of AST, ALT and ALK enzyme in the treated groups of healthy animals in AgNPs (T1) and Spirulina (T2) or both together (T3) compared with their values in The control group (T0) was at 7.13, 56.1 and 1136 IU/L, respectively.

As for the case of hyperlipidemia in laboratory rats, it caused an increase in the concentration of these enzymes in the blood serum, and the case of oral administration of both AgNPs (T5) and Spirulina (T6) or both together (T7) caused a modification of the values of AST, ALT and ALK enzymes and was AST values at 9.92, 8.23 and 8.03 IU/L respectively and ALT values at 63, 63 and 61 IU/L respectively as well as ALK values at 1292, 1216 and 1197 IU/L respectively compared with their values in The injury group (T4) was at 14,23, 81,33, 1477 IU/L for AST, ALT, and ALK, respectively.
Table 2: Effect of Oral administration of Spirulina and AgNPs particles on enzyme parameters of rat liver

<table>
<thead>
<tr>
<th>Transactions</th>
<th>AST IU/L</th>
<th>ALT IU/L</th>
<th>ALK IU/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>7.13d</td>
<td>56.1c</td>
<td>1136d</td>
</tr>
<tr>
<td>±0.88</td>
<td>±2.50</td>
<td>±18.69</td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>7.23d</td>
<td>57.6c</td>
<td>1148d</td>
</tr>
<tr>
<td>±0.76</td>
<td>±3.56</td>
<td>±14.77</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>6.83d</td>
<td>55.6c</td>
<td>1139d</td>
</tr>
<tr>
<td>±1.22</td>
<td>±5.23</td>
<td>±15.84</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>6.73d</td>
<td>54.3c</td>
<td>1143d</td>
</tr>
<tr>
<td>±1.01</td>
<td>±2.96</td>
<td>±15.05</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>14.23a</td>
<td>81.33a</td>
<td>1477a</td>
</tr>
<tr>
<td>±1.59</td>
<td>±4.33</td>
<td>±14.97</td>
<td></td>
</tr>
<tr>
<td>T5</td>
<td>9.92b</td>
<td>63b</td>
<td>1292b</td>
</tr>
<tr>
<td>±1.73</td>
<td>±2.40</td>
<td>±14.83</td>
<td></td>
</tr>
<tr>
<td>T6</td>
<td>8.23c</td>
<td>63b</td>
<td>1216c</td>
</tr>
<tr>
<td>±1.82</td>
<td>±2.32</td>
<td>±15.02</td>
<td></td>
</tr>
<tr>
<td>T7</td>
<td>8.03c</td>
<td>61b</td>
<td>1197c</td>
</tr>
<tr>
<td>±3.18</td>
<td>±1.57</td>
<td>±13.44</td>
<td></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.

The results agreed with what Oriquat (Oriquat, G. A. 2018) found in the role of Spirulina in improving the health status of fatty liver enzymes in rats fed a high-fat diet. The ALT enzyme values in the affected group were at 64.4 IU/L, while it decreased in the groups dosed with Spirulina at a high concentration 100, 200 and 300mg/kg to become at 55.8, 43.2, 37.6 IU/L respectively, while their values in AST enzyme became 144.4, 129.7, 122.5 IU/L for the same concentrations respectively compared to the injury group in which the AST value was 168.7 IU/L.

The results also agreed with Nayyef (Nayyef, S. H. 2020), which found a role for Spirulina at a concentration of 200mg/ml in reducing liver enzymes AST and ALT compared to the group infected with Staph.aureus and untreated, and also agreed with Khalil et al., (Khalil, S. R. et al., 2020) who found a positive role for Spirulina in improving The health status of liver enzymes in all groups that were dosed with Spirulina at a concentration of 300mg/kg/day and for all groups treated with furan and untreated, Li et al., (Li, T. T. et al., 2021) mentioned in a study they conducted about the importance of Spirulina in improving the health status of liver enzymes in animals fed On a high-calorie diet.

The results converged with Lee et al., (Lee, J. H. et al., 2018) that the value of AST and ALT in the control group was at 133.80, 26.40 IU/L, respectively, while the group dosed with AgNPs at a concentration of 100μg was at 111.80, 27 IU/L for the same liver enzymes respectively.

REFERENCES: