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ORIGINAL ARTICLE

Effect of Oral Dosage from *Spirulina platensis* and Silver Nanoparticles on some Blood parameters on Male Rat's Diabetics induced with alloxan

Saad D. Oleiwi

Food Science Department, College of Agriculture, Tikrit University, Tikrit, Iraq.

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Abstract

The research was conducted to identify the therapeutic ability of 150 mg/ml of *Spirulina platensis* and 10 mg/ml of silver nanoparticles (AgNPs) on some Blood parameters of male rats induced people with diabetes with alloxan after breading for 23 days. The results showed that the rats treated with alloxan (M2) had a negative significant ($p<0.05$) effect on the parameters of blood parameters compared to the control group (M1). While the rat group treated with AgNPs (M3) or *S. platensis* (M4) or both (M5) caused significantly improved levels of RBCs and became at 7.28, 7.40, 7.65 $\times 10^6/mm^3$, respectively, compared to the infected group (M2) which at 6.78 $\times 10^6/mm^3$. The Hemoglobin (Hb) and Hematocrit (Hct) values were significantly increased in the treated groups M3, M4, and M5 compared with the control group M1 at 12.34 g/dl and 42.96%, respectively, and with the same parameters at M1 and M2 that at 11.48 g/dl, 39.42 % respectively. The Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC), were increased significantly in the M2, at 18.46 pg, 31.74 g/dl, respectively, compared to the treatment groups M3, M4, and M5 and compared with M1 at 16.37 pg, 27.98 g/dl respectively. The oral dosage in treatment groups M3, M4, and M5 caused a significant decrease in the total WBCs and Lymphocytes (Lym) compared to the M2, which became 9.98 10^3 cells. /mm3, 79.62%, respectively compared with the control group M1 at 7.28 10^3 cell/mm3, 72.36 % respectively. The results also indicated that the biomarkers significantly improved in Monocytes (Mon) and Granulocytosis (Gra) in the treatment groups M3, M4, and M5 compared to the infection group M2.

1. Introduction

Diabetes is the most challenging health problem in the twenty-first century, and it is a group of metabolic disorders of the endocrine glands that result in an increase in the level of glucose in the blood (hyperglycemia) due to insufficient secretion of the hormone insulin or its ineffectiveness as a result of the body's resistance to it. The disease is associated with many diseases and the failure of many organs, such as liver damage and blindness that affects adults. It can also cause amputation of the lower limbs, and it is mainly responsible for cardiovascular diseases, stroke, high blood pressure, and nerve damage [1]. Diabetes and osteoporosis in the elderly [2], and another harmful effect is its effect on the tissues of the mouth and teeth, which leads to tooth loss [3] .*Spirulina platensis* is one of the most important nutritional supplements or natural sources used in the treatment of diabetes mellitus [4] due to its nutritional properties in addition to its antioxidant, anti-

Corresponding author: Saad D. Oleiwi
Email Address: saaddhamin3@tu.edu.iq
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inflammatory, and immune-enhancing efficacy [5]. *S. platensis* is one of the best organic foods, due to its good content of protein, fats, carbohydrates, sterols, minerals, vitamins, and many other nutrients of health importance to the body. Therefore, it is one of the best types of available and safe nutritional supplements [6]. The ability of *S. platensis* to reduce the level of sugar and fats in the blood is due to its content of Polyunsaturated Fatty acid (PUFAS) and biologically active peptides such as glutathione, which gave spirulina the ability to control the level of insulin [7,8]. Silver nanoparticles (AgNPs) belong to inorganic metal particles, the size of its nanoparticles ranges from 1-100 nm, and thus they have different chemical and physical properties than the properties of the original silver metal ions [9], which gave them importance for use in many applications and studies and in all over the world [10], as it was considered an important material for many applications due to its promising antimicrobial properties in addition to its physical and chemical properties [11], which are used in coating and wrapping medical instruments and products such as devices used in intravenous catheters as well as needles scalpels, gastroscope, and intestines [12] and tools used in dentistry [13]. And it is used as a means of transporting antibiotics, which improves its effectiveness in eliminating pathogenic microorganisms [14]. From the aforementioned, the aim of the research was to study the effect of treatment with *S. platensis* and AgNPs on male rats with diabetes mellitus induced by alloxan.

2. Materials and methods

2.1 Preparing the experimental animals

The study was conducted in the animal house of the College of Veterinary Medicine and in the laboratories of the Department of Food Sciences-College of Agriculture- University of Tikrit. In this study, 25 male white rats were used for a period of 4 weeks. Their weight ranged between 252-240 grams. They were divided randomly into five groups, and each group had five animals. They were fed during the experiment period on the standard diet. Four groups were infected with diabetes induced by alloxan at a concentration of 150 mg/kg, and one group was left without infection as a control group. The level of glucose was measured in the injected animals after 48 hours, and the animal was considered infected when the blood sugar level reached above 190 mg/dl, which appeared It has signs of infection, such as frequent urination. Diabetic animals were treated by giving 150 mg/ml *S. platensis* algae powder and 10 mg/ml AgNPs as follows: M1 control group, M2 diabetic and untreated group, M3 diabetic group treated with 10 mg/ml ml AgNPs, M4 group, diabetic group treated with 150 mg/ml of spirulina algae, M5 group, diabetic group treated with 10 mg/ml + 150 mg/ml of *S. platensis* algae powder.

2.2 Blood sample collection

At the end of the experimental period, the animals were anesthetized by chloroform, then blood samples were drawn directly from the heart using the Cardiac Puncture method. The amount of blood taken was between 0.5-1 ml and placed in tubes containing EDTA anticoagulation to measure Complete Blood Pictures (CBC), which were performed within less than an hour after the blood was withdrawn [15].

2.3 Complete blood Picture (CBC)

The level of Red blood cells (RBCs), white blood cells (WBCs), Hemoglobin (Hb), Monocytes (Mon), Lymphocytes (Lym), Granulocytosis (Gra), Hematocrit (Hct), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and Platelets (PLT) which were measured using the Hematology Analyzer (French origin).

2.4 Statistical analysis

Use the ready-made statistical program SAS [16] and to find out the significant differences between the different means at a probability level of 0.05, use the Duncan test [17].

3. Results and discussion

3.1 Effect on Complete Blood Picture

Table 1 shows the effect of oral dosage of *S. platensis* and AgNPs on the Complete Blood Picture of alloxan-induced diabetic rats. The results showed that there was a significant decrease $p<0.05$ in the total number of Red Blood Cells (RBCs) for the untreated group M2, and it was $6.78 \times 10^6/mm^3$ compared with the numbers of the control group M1, which was at $7.86 \times 10^6/mm^3$. As for the diabetic groups treated with AgNPs (M3) or treated with Spirulina (M4), there was an improvement, as it increased significantly compared to the infection group, where the number of RBCs reached 7.28, 7.40 x 106/mm3, respectively, and this indicates a positive improvement, as it increased significantly. Compared with the infection group (M2), but it remained significantly lower compared to the control group (M1). As for the group with diabetes treated synergistically with spirulina and AgNPs (M5), there was no significant difference between it and the control group (M1). As for the level of Hemoglobin (Hb), oral administration of *S. platensis* and AgNPs improved the health status of diabetic animals, as the results were significantly identical in the infected group given AgNPs (M3), the infected group given Spirulina (M4), and the infected group given *S. platensis* and AgNPs. Combined (M5) with the control group (M1), which amounted to 12.34 g/dl, while the untreated group (M2) had a significant decrease in its values, amounting to 11.48 g/dl. The same was the case concerning the percentage of Hematocrit (Hct), the percentages were significantly identical $p<0.05$ in all groups with diabetes and treatment, namely the infected group given AgNPs (M3), the infected group given Spirulina (M4), and the infected group given *S. platensis* and AgNPs together (M5) with the control group M1

which amounted to 42.96 %, and on this basis, the M3, M4, and M5 groups had a significant increase in their values compared to the diabetic and untreated group M2 which was at 39.42 %. Concerning Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC), the results showed that the case of diabetes mellitus (M2) showed stress on animal cells and increased their values significantly, as they were at 18.46 pg and 31.74 g/dl respectively compared to the control group (M1) in which the values of MCH and MCHC were at 16.37 pg and 27.98 g/dl respectively, the condition of oral administration to diabetic rats of either AgNPs (M3) or Spirulina (M4) or both (M5) caused the modification Positive values for each of the MCH and MCHC values were significantly identical to the control group (M1), except for the infected group treated with Spirulina (M4), whose value was significantly decreased in MCHC compared to the control group.

Table 1: Effect of orally dosage of Spirulina and AgNPs on Blood Picture parameters of diabetic rats

groups	RBCs ($\times 10^6/mm^3$)	Hb g/dl	Hct %	MCH pg	MCHC g/dl
M1	7.86a ±0.18	12.34 a ± 0.47	42.96a ± 1.33	16.37b ± 0.36	27.98b ± 0.71
M2	c6.78 ± 0.19	b 11.48 ± 0.27	b 39.42 ± 0.92	a18.46 ± 0.57	a 31.74 ± 0.58
M3	b7.28 ± 0.18	a 12.48 ± 0.25	a42.93 ± 1.20	b16.43 0.53	b 28.43 ± 0.20
M4	b7.40 ± 0.19	a 12.15 ± 0.95	a 43.80 ± 2.50	b 16.70 ± 0.80	c 27.50 ± 0.50
M5	a7.65 ± 0.76	a 12.56 ± 1.39	a 43.83 ± 4.95	b16.31 ± 0.35	b 28.20 ± 0.20

Different letters in the same column indicate significant differences at the probability level of 0.05 ± = standard error. Averages are for five animals. M1: control, M2: induced diabetes without treatment group, M3: infected and treated group given AgNPs, M4: infected and treated group given Spirulina, M5: infected and treated group given AgNPs with spirulina.

It was clear from the results in 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, and this reflects the positive effect of silver nanoparticles and spirulina powder on those parameters and thus on the health of the animals.

The results agreed with Al-Samarray [18], which found a role for spirulina in the treatment of Complete Blood Picture and the return of values for each of the RBCs, Hb, and Hct to a level close to the normal level in mice with induced diabetes and treated with spirulina. The therapeutic role of spirulina is due to its content of vitamin B6, which was found to have an important role in the formation and regeneration of blood cells [19]. The results also agreed with Bashir et al. [20] who noticed 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, Hct, and this gives an impression of the importance of spirulina in Treatment of blood diseases and the need to use them in the treatment of malnutrition.

Spirulina is one of the rich and beneficial foods for human and animal health, due to its content of many active elements that have been found to have a role in improving the health of CBC an example of effective compound is that it contains protein of type C-phycocyanin which has proven its positive role in improving the level of Hemoglobin and RBCs, Hct, MCH, and MCHC [21]. The results converged with Lee et al. [22] who found that the values of blood images for the same study RBCs, Hb, Hct, MCH, and MCHC, did not differ significantly in rats dosed with silver nanoparticles from the control sample. The results agreed with AL-Daami [23] who noted the importance of AgNPs in improving the health status of red blood cell count and hemoglobin in alloxan-induced diabetic rats. The role of AgNPs in improving the health status of blood images may be due to their ability to stimulate some of the mechanisms that produce blood cells.

3.2 Effect of orally dosage of Spirulina and AgNPs on total and differential numbers of White Blood Cells

The effect of oral administration of spirulina and silver nanoparticles (AgNPs) alone or together in diabetic laboratory rats on the total and differential number of white blood cells was shown in Table (2). The results showed that the oral administration of AgNPs (M3) and Spirulina (M4) to diabetic animals alone or together (M5) decreased significantly ($p<0.05$) the total numbers of WBCs compared to the untreated group (M2), which Its value was at 9.98 10^3cell/mm^3, while the treatment groups (M3, M4, M5) agreed significantly with the control group M1 which amounted to 7.28 10^3 cell/mm^3. 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 infected and treated groups (M3, M4, M5) compared with the percentage of their presence in the control group (M1), whose percentage was 72.23%. All groups were significantly decreased compared with their value in the group of diabetes mellitus (M2) in which the values of Lymphocytes were at 79.62%, and this reflects the positive effect of spirulina and AgNPs on the health and immunological status of rats. As for Monocytes (Mon) cells, there was no significant difference between the group given AgNPs (M3) or spirulina (M4) or given from both (M5) with the control group

(M1) in which the proportion of Mon was at 4.68%, and thus it increased significantly compared to the untreated infection group (M2), in which the percentage of Mon was at 3.38%. The percentage of Granulocytosis (Gra) decreased in the M2 infection group and was at 12.80%, while it usually increased significantly in the group given Spirulina M4 and the group given AgNPs with Spirulina M5, where no significant difference was indicated between them and the M1 control group, whose percentage was at 17.94%, except for the group given AgNPs were significantly increased compared to the M2 infection group and decreased compared to the M1 control group, where the Gra ratio in the M3 group was 16.76%. The role of spirulina was positive in reducing oxidative stress by reducing dependence on the immune system, as spirulina reduced inflammation in the cells of the body, and thus it is effective for treating diabetes, obesity, and related disorders through its role in reducing appetite [24]. The results agreed with what was mentioned by Bashir et al. [20] who gave spirulina to rats as a diet to observe its effect on them, as they mentioned that there were no significant differences between the control group and the rest of the groups given spirulina with regard to the number of white blood cells (WBCs) and the same was applied to Lym and Mon, The results agreed with Grover et al. [21] who found a positive role for spirulina in improving the health status of mice dosed with spirulina on lymphocytes and monocytes, and this was attributed to the content of spirulina of C-phycocyanin protein, which was attributed to the ability of spirulina to resist oxidative stress and increase immunity to infectious diseases.

The results agreed with Raheem [25], who noted that the values of WBCs, Lym, and Gra remained within the normal limits for rabbits dosed with AgNPs at a concentration of 50 µg compared to their numbers in the undosed control group, while the percentage of MON increased in the dosed group compared to the control group. The results converged with what was found by Lee et al. [22] in that the number of white blood cells and the percentage of lymphocytes and monocytes did not differ significantly in laboratory animals injected with AgNPs at a concentration of 10 and 100 µg compared to their numbers in the control group, where there was a decrease but not significant in the totals dose compared to the preparation in the control group. The results agreed with AL-Daami [23] who observed a decrease in the number of white blood cells (WBCs) in the induced diabetic groups treated with AgNPs compared with the untreated group, as the WBC concentration reached 8.42, 11.87 10^3cells/mm^3, respectively. The ability of AgNPs to reduce the number of white blood cells is due to their ability to resist oxidative stress resulting from diabetes, as having this disease enhances the immune system to produce more white blood cells [26].

Table 2. Effect of oral dosage of Spirulina and AgNPs on the Total and Differential number of White Blood Cells

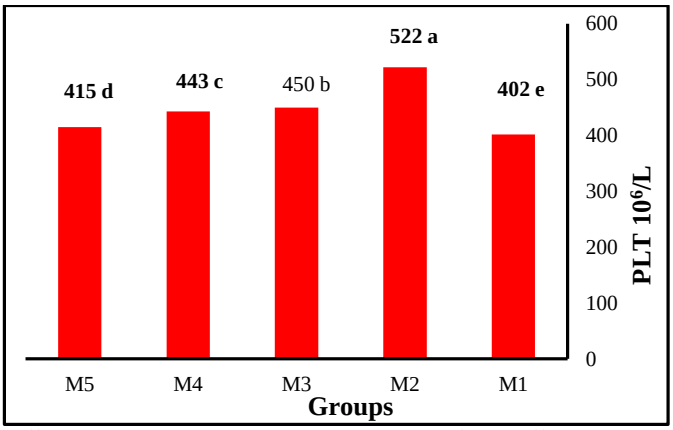
Groups	WBCs 10^3 cell/mm^3	Lym %	Mon %	Gra %
M1	7.28 b ±0.27	72.36 b ±1.47	4.68 a ±0.97	17.94 a ±2.31
M2	a9.98 ±3.87	a 79.62 ±4.71	3.38 b ±0.41	12.80 c ±1.05
M3	b7.80 2.02	b 73.40 ±5.37	4.23a ±0.86	16.76a ± 2.64
M4	b7.05 ±4.15	71.95 b ±4.45	4.15 a ±1.15	18.10 a ±1.60
M5	b6.97 ±1.99	72.40 b ±3.38	4.60 a ±0.88	17.20 a ±1.15

Different letters in the same column indicate significant differences at the probability level of 0.05 ± = standard error. Averages are for five animals. M1: control, M2: induced diabetes without treatment group, M3: infected and treated group given AgNPs, M4: infected and treated group given Spirulina, M5: infected and treated group given AgNPs with spirulina.

3.3 Effect of orally dosage of spirulina and AgNPs on platelets

Platelets PLT are considered one of the main components of the blood, and their function lies in the formation of thrombi to prevent bleeding in the blood vessels, and the occurrence of a decrease or increase in the level of these platelets causes thrombocytopenia, so they are an indicator of the health status of the organism. The results showed in Figure 1 that the number of platelets in the blood of diabetic rats given AgNPs (M3) or Spirulina (M4) or both (M5) had a significant decrease ($p<0.05$) in their numbers and they were at 450, 443, 415 10^6/L, respectively, compared to their numbers in the M2 diabetic group, which was at 522 106/L, the condition of diabetic rats caused an increase in the number of platelets, so all groups (M2, M3, M4, M5) gave a significant increase compared to the control group which was at 402 10^6/L. The results agreed with Hassan et al. [27] who found a role for spirulina in reducing the value of blood platelets in animals suffering from lead poisoning, as the dose for 60 days caused the PLT values in the treated group to be 515 10^6/L in male rats, while in females Rats and for the same study, their values were at 445, 376 10^6/L for both the lead-infected group and the treated group, respectively. The results converged with the study of Krutyakov et al. [28] who noted that there were no significant differences between the group dosed with silver nanoparticles at a concentration of 2.25 mg with the control

group whose values were at 734, 732 10^6/L, respectively. The reason for the decrease in the number of blood platelets may be due to the ability of spirulina algae and silver nanoparticles to resist oxidative stress and the bad metabolic symptoms caused by diabetes.



Different letters in the same column indicate significant differences at the probability level of 0.05 ± = standard error. Averages are for five animals. M1: control, M2: induced diabetes without treatment group, M3: infected and treated group given AgNPs, M4: infected and treated group given Spirulina, M5: infected and treated group given AgNPs with spirulina

Figure 1: Effect of oral administration of spirulina algae and AgNPs on platelet count PLT (10^6/L) in serum of diabetic rats.

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