Effect of oral dosage of *Spirulina platensis* and silver nanoparticles on glucose, lipid profile, and liver enzymes in male rats induced diabetics by alloxan

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Abstract

Diabetes is believed to be one of the most important challenges that are facing societies around the world. The research was conducted to determine the effectiveness of 150 mg/ml of *Spirulina platensis* and 10 mg/ml of silver nanoparticles Ag-NPs on the sugar level, lipid profile, and liver enzyme concentration in male rats. Diabetes was induced with alloxan and bred for four weeks. The rats were divided randomly into five groups and every single group contained five rats; M1 was the control group, M2 was a treated diabetic rat, M3 treated diabetic rats with 10 mg/ml Ag-NPs, M4 treated diabetic rats with 150 mg/ml *S. platensis* and M5 group with diabetic rats that were treated with 10 mg/ml Ag-NPs + 150 mg/ml *S. platensis.* The results showed that the group of rats infected with Alloxan (M2) caused negative effects at a significant level (P<0.05) on the blood sugar level, while the group of rats treated with Ag-NPs (M3) or *S. platensis* (M4) or both (M5) showed a positive effect on the blood glucose rate, which reached 292.6, 210.5, and 199.3 mg/dl, respectively, compared to the (M2) group, which was 441.8 mg/dl. The results of the lipids profile, the group with diabetes (M2) showed an increase in the level of triglycerides (TG), cholesterol (TC), low-density lipoproteins (LDL), and very low-density lipoproteins (VLDL), and a decrease in the level of high-density lipoproteins (HDL). The treatment groups M3, M4, and M5 improved significantly positive blood lipid levels. The same situation applied to the liver enzymes AST, ALT, and ALP, whose values decreased significantly in the treatment groups (M3, M4, and M5) compared to the infection group (M2).

Keywords: *Spirulina platensis*, Ag-NPs, Diabetes, Blood glucose, Lipid profile, Liver enzymes.

Introduction

Diabetes is the most challenging health problem of the twenty-first century. According to the International Diabetes Federation, diabetes currently affects nearly half a billion people around the world, with 451 million people suffering from diabetes,

which by 2045 is expected to rise to 693 million [1,2] Diabetes is a group of metabolic disorders that occur in the endocrine glands and result in high levels of glucose in the blood This is due to insufficient secretion of the insulin or its ineffectiveness as a result of the body's resistance to the insulin hormone. Diabetes causes many diseases, disorders, and failure of many organs, such as liver damage and blindness, which mainly affects adults. It is the main responsible for heart and blood vessel diseases, high blood pressure, and nerve damage, and stroke [3].

Spirulina platensis is one of the most important nutritional supplements that come from natural sources and is used in the treatment of diabetes, because this alga possesses nutritional and therapeutic properties that give *S. platensis* an important role as an antioxidant, anti-inflammatory, and immune booster [4]. *S. platensis* is one of the most prominent microalgae that contain important nutrients in a comprehensive manner that has been used as a functional food as well as for therapeutic and pharmaceutical applications, Spirulina was widely produced and used as a nutritional supplement to treat malnutrition and enhance immune functions, in addition to treating many diseases [5,6]. The ability of *S. platensis* to lower blood sugar levels is due to its content of biologically active peptides such as glutathione, which gave Spirulina has the ability to control insulin and blood sugar levels and thus maintain a healthy blood sugar level [7,8].

Ag-NPs belong to inorganic mineral particles, the size of their nanoparticles is 1-100 nm, and thus they possess physical and chemical properties different from the properties of the basic silver metal ions [9], which gave them importance for use in many applications and studies all over the world [10].

In addition to its important role as an antimicrobial due to its chemical and physical properties, [11], as it was used in the manufacture of wound dressings and was used in the treatment of diabetic foot [12]. It was also used as a means of transporting antibiotics, which improved its effectiveness in eliminating pathogenic microorganisms [13]. Beside that it has a role in diabetes treatment [14].

The research aimed to study the effect of treatment with *S. platensis* and Ag-NPs on male rats with alloxan-induced diabetes.

Materials and Methods
Experimental animals

The study was conducted in the "animal house" of the Veterinary Medicine College and in the laboratories of the Food Science Department, College of Agriculture - Tikrit University. 25 white male rats were used in this study for a period of 23 days, in addition to 10 days during which the animals were left to adapt to their new place, and their weight was It ranged between 240-252 grams and was randomly distributed into five groups, each group had five animals, and they were fed the standard diet during the experimental period.

Four groups of them developed diabetes induced by Alloxan at a concentration of 150 mg/kg, and one group was left unaffected as a control group. The sugar level in the injected animals was measured after 48 hours, and the animal was considered

infected when the blood sugar level reached higher than 190 mg/dl according to Adeyi *et al.*, [15] which appeared a signs of infection, such as frequent urination and lethargy. Diabetic animals were treated by giving them 150 mg/ml of *S. platensis* algae powder and 10 mg/ml of Ag-NPs (The given concentrations were determined according to Oleiwi, [16]), as follows: control group M1 (nothing was given), group M2, a group with diabetes and untreated, group M3, a group with diabetes and treated with 10 mg/ml Ag-NPs, group M4 a group with diabetes and treated with 150 mg/ml of spirulina algae, M5 group group with diabetes mellitus treated with 10 mg/ml of Ag-NPs + 150 mg/ml of *S. platensis* algae powder.

Collection of Blood Sample

At the end of the experimental period, the rats were anesthetized by the "chloroform", after that, the blood samples were drawn from the heart directly using the "Cardiac Puncture method". A quantity between 3- 3.5 ml of the animals blood was withdrawn, which was collected in a test tubes free of "anticoagulants" and left for a 15 mint., at the room temperature, after that serum was separated using centrifugation at 3000 rpm for 15 minutes and was kept at (-18) ℃ in sterile plastic tubes until biochemical measurements were performed [17].

Glucose Concentration Determination

The glucose concentration in the serum of rats blood was evaluated by using a ready-made quantifying measuring kit from the (Spanish company, Bio Systems) [17].

Determination of Lipid Profile

The level of triglycerides (TG), cholesterol, Very Low-Density Lipoprotein (VLDL) and High-Density Lipoprotein (HDL) were evaluated using a ready-made analysis reagent that manufactured by HUMAN diagnostic worldwide company (Germany) according to that mention in Titez [17], Except for Low Density Lipoproteins (LDL), they were estimated according to the following equation:

"LDL-c (mg / dl) = Total cholesterol- (HDL-c + VLDL-c)" [18]

Liver Enzyme Activity evaluation

A ready-made assay kit manufactured by the German company HUMAN diagnostic worldwide was used to measure quantitatively the activity of the enzyme Alkaline phosphatase (ALP), Alanine amino transaminase (ALT) and Aspartate amino transaminase (AST) in the serum of the rats blood, according to the kinetic method used by Titez [17].

Statistical analysis

The complete randomizes design system was used to analyze the results and estimate the significant differences between the means from the groups of the experiment with an emphasis on these differences by extracting the Standard error (SE), Statistical analyzes were performed at a significant level (P≤0.05) [19]. And the means were compered by the use of Duncan test to extract the differences between different study groups [20].

Results and Discussion

Effect of oral dosage of *S. platensis* and Ag-NPs on Blood sugar

Table 1 shows the effect of oral administration of spirulina algae or Ag-NPs, individually or together, on the blood glucose level in rats with alloxan-induced diabetes. The results showed that the highest significant value recorded at the probability level P <0.05 belonged to the untreated diabetes group, which had a blood sugar value of 441.8 mg/dl.

While the values began to decrease significantly in the treated infected groups compared to the infection group, they remained significantly higher (P<0.05) than the control group (M1), where the values of the treated groups remained at 292.6, 201.5, and 199.3 mg/dl for the groups treated with Ag-NPs particles (M3), and spirulina (M4) or both (M5), respectively.

Table (1): The effect of oral dosage of spirulina algae and Ag-NPs on the blood sugar level in groups of rats with alloxan-induced diabetes (mg/dl).

Groups	M1	M2	M3	M4	M5
Blood sugar	96.4 e	441.8 a	292.6 b	210.5 c	199.3 cd
Mg/dl	± 3.8	± 5.5	± 4.3	3.5	± 1.8

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

M1: Control, M2: Induced diabetes group without treatment, M3: Infected and treated group given Ag-NPs, M4: Infected and treated group given spirulina, M5: Infected and treated group given Ag-NPs with spirulina.

The results showed the healthy role of spirulina in treating diabetes and high blood glucose. The results were consistent with Layam and Reddy [21] who noticed a decrease in the blood glucose level of diabetic rats treated with spirulina at a concentration of 5, 10, 15 mg/kg, as the blood sugar concentration reached this level totals were at 232.22, 220.8, 159.5, and 114 mg/dl in the infected and treated groups with concentrations of 5, 10, and 15 mg/kg, respectively.

The results also agreed with Lee *et al.,* [22], whose study included giving spirulina algae to a group of people suffering from high blood sugar for a period of 12 weeks, through which they found the ability of spirulina to maintain blood sugar within normal limits. Abdel-Daim *et al.,* [23] also stated that dosing laboratory rats with spirulina algae at a concentration of 500 mg/kg per day significantly reduces the level of blood glucose and improves the health condition of the animals. The blood sugar concentration in the untreated infected group and the infected group that was treated reached at 258.6 and 154 mg/dl, respectively.

Spirulina have the ability of controlling diabetes, this is due to it containing unsaturated fatty acids and many biologically active peptides [8]. Hu *et al.,* [24] identified some types of these peptides, which they classified into the following names and types: GVPMPNK, RNPFVFAPTLLTVAAR, and LRSELAAWAR, where the ability of these peptides to inhibit some enzymes responsible for raising blood sugar, such as α-amylase and α-glucosidase, was found.

Regarding treatment with Ag-NPs, the results of the study were consistent with AL-Daami [25], who found that diabetic rats treated with biosynthesized Ag-NPs had a role in lowering blood sugar, and the results agreed with Prabhu *et al.,* [26], who found that dosing diabetic rats with Diabetes using Ag-NPs at a concentration of 10 mg/kg would lower blood sugar levels.

The role that Ag-NPs play important role in controlling blood glucose is due to their ability to inhibit some of the enzymes responsible for high blood sugar, such as α-amylase and α-glucosidase [27,28].

Effect on Blood Lipid profile parameters

Table 2 shows the effect of oral administration of Ag-NPs and *S. platensis* on the blood lipid profile in male diabetic rats. The results showed a significant increase ($P<0.05$) in the levels of Triglycerides (TG), Cholesterol (TC) and low-density lipoproteins (LDL) in the untreated infection group. (M2) whose concentrations reached 254, 164, and 88.60 mg/dl, respectively, compared to the control group, who's TG, TC, and LDL values were 149, 102, and 38.2 mg/dl, respectively.

The results agreed with Mans and Aburjai [29] who noticed an increase in the blood fat profile in diabetic animals, and this was attributed to several activities that may occur in the affected animal, including a decrease in the effectiveness of the Lipoprotein Lipase (LPL) enzyme due to a lack of insulin, and this enzyme has a major role in analyzing Triglycerides into glycerol and fatty acids [30], Also, the inability of the diabetic body to consume blood glucose as a source of energy results in the stimulation of lipolysis in adipose tissue and thus an increase in fatty acids, which leads to an increase in cholesterol in the blood serum [31].

The results showed that the concentration of TG, TC and LDL in the group treated with spirulina and Ag-NPs began to decrease significantly ($P<0.05$) in the treatment groups compared to the infection group, thus approaching that of the control group (M1). The concentration of TG in the groups treated with Ag-NPs (M3) reached or Spirulina (M4) or both were at 171, 185, and 148 mg/dl, respectively. The TC values were at 130, 126, and 125 mg/dl for the same groups, respectively, and the LDL values were at 59.5, 53.2, and 46.1 for the same groups, respectively. The same was the case in the case of Very Low-Density Lipoprotein (VLDL) An improvement was observed in the values of the groups treated with spirulina and silver nanoparticles, individually or together, compared to the untreated infection group (M2). Table 1 also shows the values of high-density lipoprotein (HDL), which recorded the least significant decrease in the infection group (M2), amounting to 24.6 mg/dl compared to the control group (M1), amounting to 33.7 mg/dl. The results agreed with Achi *et al.,* [32] about their

study on rats induced diabetes, the reason for the reduction in HDL-c in diabetes is due to the amplified activity of the enzyme "cholesterol ester transferase" which works to transfer cholesterol esters from HDL to VLDL, leaving HDL rich in triglycerides and less affinity for Apo-A, so it remains free and becomes easier to apply through kidney.

The case of oral administration to diabetic rats with both Ag-NPs (M3) and, *S. platensis* (M4) or their mixture together (M5) caused its value to be adjusted to be at 36.3, 35.8, and 49.3 mg/dl, respectively, as it decreased significantly compared to the infection group. (M5).

Table (2): Effect of oral dosage of both spirulina and Ag-NPs on blood lipid profile (mg/dl).

Groups	TG mg/dl	TC mg/dl	LDL mg/dl	VLDL mg/dl	HDL mg/dl
M1	149d ±4.11	102d ± 7.2	38.2e ±1.3	29.8d ± 2.4	33.7c ± 2.68
M2	254a ±5.07	164a ±9.45	88.60a ±1.07	50.80a ±4.01	24.6d ±1.07
M3	171c ±3.09	130b ±7.93	59.5b ±3.75	34.2c ±1.25	36.3b ±2.20
M4	185 b ±9.50	126c ±6.4	53.2c ±4.10	37b ±2.90	35.8b ±2.20
M5	148d ±8.83	125c ±6.88	46.1d ±1.58	29.6d ±1.36	49.3a ±2.69

Different letters in the same column indicate significant differences at the probability level of ±0.05 = standard error. Averages are taken for five animals.
M1: Control, M2: Induced diabetes group without treatment, M3: Infected and treated group given Ag-NPs, M4: Infected and treated group given spirulina, M5: Infected and treated group given Ag-NPs with spirulina.

The results agreed with Lee *et al.,* [33] who found, through a study conducted on humans suffering from high blood sugar, a role for spirulina in improving the health status of blood lipid profile, and the results agreed with Gargouri *et al.,* [34] who found a role for spirulina algae supplements in lowering blood lipid profile of harmful cholesterol and improve the level of beneficial cholesterol in diabetic rats, the reason was explained by the ability of spirulina to reduce the absorption of cholesterol in the intestine, in addition to its content of the amino acid cysteine found in the protein C-Phycocyanins. On the other hand, the results were in line agreement with [35,36] how found through a study on humans how are suffering from diabetes type II how were supplemented with spirulina sauce every day as a treatment and they found a reduction in TG, TC and LDL when compared with their level in the beginning of the experiment and they observed reduction in waistline and hungriness and increase the feeling of fullness among patients.

The role of spirulina algae in treating diabetes, reducing harmful fats in blood and raising beneficial lipids can occur by controlling the level of glucose in the blood. Spirulina is also rich in unsaturated fatty acids, which rid the body of saturated acids and sugars to replace them, in addition to spirulina containing significant amounts of vitamin C and E, which are natural antioxidants Which service to remove free radicals and reduce the oxidation process that raises harmful lipids [37,38].

The results also agreed with Kaur *et al.,* [39] who indicated a decrease in the values of TC, TG, LDL, and VLDL in rats given Ag-NPs compared to the control group fed a high-fat diet, and the results agreed with the study conducted by Al-Dujaili and Al-Shemeri [40] who found a role for silver nanoparticles in improving the health status of blood lipid profile through a study they conducted on rats suffering from high blood lipids. Over all in this study, the results of this study reach an agreement with UI Haq *et al.,* [41] how found that Ag-NPs have a major role in improving the health state of rats with induced diabatic with alloxan by reduction of TC, TG and LDL in diabetic and treated rats when compared with its values before treatment. The reason for the ability of nanoparticles to reduce blood fats, especially cholesterol, may be due to their ability to inhibit the mechanisms responsible for the absorption of cholesterol in the body [42,43].

Effect on liver enzymes

Table 3 shows the effect of oral dosage of spirulina algae and silver nanoparticles on rat liver enzyme parameters. The results showed that the diabetic status of laboratory rats caused a significant ($P<0.05$) increase in the concentration of these enzymes in the blood serum, which reached the values of the AST and ALT enzymes and ALP in the infected group (M2) at 12.50, 75.2, 1397 IU/L, respectively compared to the control group (M1), in which the values of the same enzymes were 7.46, 57.4, and 1127 IU/L, respectively. Oral dosage of Ag-NPs (M3) and spirulina (M4) or both (M5) caused a positive modification of the values of the liver enzymes AST, ALT, and ALP, as the AST values for these groups were at 8.73, 8.05, and 7.80 IU/L, respectively. ALT values were 64.3, 68.5, and 62.6 IU/L, respectively. Likewise, the ALP values for the same groups were 1207, 1157, and 1104 IU/L, respectively, as the enzyme values decreased significantly compared to the infection group (M2).

The results agreed with Aissaui *et al.,* [44] who indicated a significant decrease in the values of liver enzymes in diabetic rats treated with spirulina algae, as spirulina's high content of antioxidant activities and its ability to remove the inhibitory effect of free radicals made it an effective treatment for high blood sugar and the liver's protection from it. The results also agreed with Gargouri *et al.,* [34] who noted the important therapeutic role of spirulina algae in reducing liver enzyme values in diabetic animals treatment with spirulina algae compared to the infected and untreated group, as the spirulina content of vitamins, especially vitamin B12, minerals, and antioxidant compounds such as B-carotene, C-phycocyanins, and proteins, contributes to the regeneration of liver cells and restoring them to their health, and the secretions of liver

enzymes returned to their normal limits, which reflects the ability of this disease. Algae help inhibit the toxic effect of alloxan on the body's cells.

Table (3): Effect of oral dosage of spirulina algae and Ag-NPs on enzymatic parameters of rat liver.

Groups	AST IU/L	ALT IU/L	ALP IU/L
M1	7.46c ±1.38	57.4d ±1.20	1127d ±16.54
M2	12.50a ±1.04	75.2a ±2.35	1397a ±12.91
M3	8.73b ±1.77	64.3c ±4.44	1207b ±18.78
M4	8.05b ±1.15	68.5b ±2.50	1157c ±13.12
M5	7.80bc ±0.75	62.6c ±3.28	1104e ±18.04

Different letters in the same column indicate significant differences at the probability level of ±0.05 = standard error. Averages are taken for five animals.
M1: Control, M2: Induced diabetes group without treatment, M3: Infected and treated group given Ag-NPs, M4: Infected and treated group given spirulina, M5: Infected and treated group given Ag-NPs with spirulina.

The results were consistent with Prabhu *et al.,* [26] who studied the abilities of silver nanoparticles to reduce the values of liver enzymes in animals with diabetes compared to the infected and untreated group, as the values of AST, ALT, and ALP enzymes were at 82.23, 54.86, and 91.32 U/L, respectively. While dosing animals with Ag-NPs particles at a concentration of 10 mg/kg reduced the values of liver enzymes to 55.34, 33.56, and 69.32 U/L for the same enzymes, respectively, the ability of Ag-NPs to reduce liver enzymes is due to their ability to inhibit Enzymes responsible for raising blood sugar levels. The results also agreed with Lee *et al.,* [33], in which the AST and ALT values in the control group were 133.80 and 26.40 IU/L, respectively, while the group dosed with Ag-NPs was at a concentration of 100 micrograms. At 111,80 and 27 IU/L for the same liver enzymes, respectively.

References

1) Thomas, R. L., Halim, S., Gurudas, S., Sivaprasad, S., & Owens, D. R. (2019). IDF Diabetes Atlas: A review of studies utilising retinal photography on the global prevalence of diabetes-related retinopathy between 2015 and 2018. *Diabetes Research and Clinical Practice*, 157, 107840.

2) American Diabetes Association. (2021). 2. Classification and diagnosis of diabetes: Standards of medical care in diabetes—2021. *Diabetes Care*, 44(Supplement_1), S15-S33.

3) Sharma, P., Hajam, Y. A., Kumar, R., & Rai, S. (2022). Complementary and alternative medicine for the treatment of diabetes and associated complications: A review on therapeutic role of polyphenols. *Phytomedicine Plus*, 2(1), 100188.

4) Seyidoglu, N., Gurbanli, R., KÖSELİ, E., Cengiz, F., & Aydin, C. (2019). The effects of *Spirulina* (*Arthrospira*) platensis on morphological and hematological parameters evoked by social stress in male rats. *Journal of Istanbul Veterinary Sciences*, 3(1), 21-27.

5) Gabr, G. A., El-Sayed, S. M., & Hikal, M. S. (2020). Antioxidant activities of phycocyanin: A bioactive compound from *Spirulina platensis*. *Journal of Pharmaceutical Research International*, 32(2), 73-85.

6) Bortolini, D. G., Maciel, G. M., Fernandes, I. D. A. A., Pedro, A. C., Rubio, F. T. V., Branco, I. G., & Haminiuk, C. W. I. (2022). Functional properties of bioactive compounds from *Spirulina* spp.: Current status and future trends. *Food Chemistry: Molecular Sciences*, 5, 100134.

7) Wan, X. Z., Li, T. T., Zhong, R. T., Chen, H. B., Xia, X., Gao, L. Y., … & Zhao, C. (2019). Anti-diabetic activity of PUFAs-rich extracts of *Chlorella pyrenoidosa* and *Spirulina platensis* in rats. *Food and Chemical Toxicology*, 128, 233-239.

8) Lafarga, T., Fernández-Sevilla, J. M., González-López, C., & Acién-Fernández, F. G. (2020). *Spirulina* for the food and functional food industries. *Food Research International*, 137, 109356.

9) Korkmaz, N., Ceylan, Y., Taslimi, P., Karadağ, A., Bülbül, A. S., & Şen, F. (2020). Biogenic nano silver: Synthesis, characterization, antibacterial, anti-biofilms, and enzymatic activity. *Advanced Powder Technology*, 31(7), 2942-2950.

10) Tortella, G. R., Rubilar, O., Durán, N., Diez, M. C., Martínez, M., Parada, J., & Seabra, A. B. (2020). Silver nanoparticles: Toxicity in model organisms as an overview of its hazard for human health and the environment. *Journal of Hazardous Materials*, 390, 121974.

11) Choudhury, H., Pandey, M., Lim, Y. Q., Low, C. Y., Lee, C. T., Marilyn, T. C. L., … & Gorain, B. (2020). Silver nanoparticles: Advanced and promising technology in diabetic wound therapy. *Materials Science and Engineering: C*, 112, 110925.

12) Hasan, S. (2015). A review on nanoparticles: Their synthesis and types. *Research Journal of Recent Sciences*, 2277, 2502.

13) Torabian, F., Akhavan Rezayat, A., Ghasemi Nour, M., Ghorbanzadeh, A., Najafi, S., Sahebkar, A., … & Darroudi, M. (2022). Administration of silver nanoparticles in diabetes mellitus: A systematic review and meta-analysis on animal studies. *Biological Trace Element Research*, 200(4), 1699-1709.

14) Adeyi, A. O., Idowu, B. A., Mafiana, C. F., Oluwalana, S. A., Ajayi, O. L., & Akinloye, O. A. (2012). Rat model of food-induced non-obese-type 2 diabetes mellitus: Comparative pathophysiology and histopathology. *International Journal of Physiology, Pathophysiology and Pharmacology*, 4(1), 51.

15) Oleiwi Saad, D. (2022). Evaluation of the efficiency of Spirulina and silver nanoparticles on the biological parameters of male rats with experimental hyperlipidemia. Tikrit University, College of Agriculture. PhD Thesis.

16) Burtis, C. A., Ashwood, E. R., & Bruns, D. E. (2021). *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*, 2006. Elsevier Inc, 702-708.

17) Andreoli, T. E., Carpenter, J., & Griggs, R. C. (2001). *Cecil essentials of medicine: Disorder of lipid metabolism*. Herbert PN Philadelphia WB Saunders company. London, Toronto, 16, 526-532.

18) Morgan, T. M., & Case, L. D. (2013). Conservative sample size determination for repeated measures analysis of covariance. *Annals of Biometrics & Biostatistics*, 1(1).

19) Duncan, D. B. (1955). Multiple range and multiple F tests. *Biometrics*, 11(1), 1-42.

20) Layam, A., & Reddy, C. L. K. (2006). Antidiabetic property of spirulina. *Diabetologia Croatica*, 35(2), 29-33.

21) Lee, E. H., Park, J. E., Choi, Y. J., Huh, K. B., & Kim, W. Y. (2008). A randomized study to establish the effects of spirulina in type 2 diabetes mellitus patients. *Nutrition Research and Practice*, 2(4), 295-300.

22) Abdel-Daim, M. M., Shaaban Ali, M., Madkour, F. F., & Elgendy, H. (2020). Oral *Spirulina platensis* attenuates hyperglycemia and exhibits antinociceptive effect in streptozotocin-induced diabetic neuropathy rat model. *Journal of Pain Research*, 2289-2296.

23) Hu, S., Fan, X., Qi, P., & Zhang, X. (2019). Identification of anti-diabetes peptides from *Spirulina platensis*. *Journal of Functional Foods*, 56, 333-341.

24) AL-Daami, Qasim J. F. (2018). Green synthesis of silver nanoparticles using a nutshell of radish extract and evaluation of its antioxidant diabetic rats activity in induced. (Doctoral Dissertation, College of Science, University of Babylon).

25) Prabhu, S., Vinodhini, S., Elanchezhiyan, C., & Rajeswari, D. (2018). Retracted: Evaluation of antidiabetic activity of biologically synthesized silver nanoparticles using *Pouteria sapota* in streptozotocin-induced diabetic rats. *Journal of Diabetes*, 10(1), 28-42.

26) Jini, D., & Sharmila, S. (2020). Green synthesis of silver nanoparticles from *Allium cepa* and its in vitro antidiabetic activity. *Materials Today: Proceedings*, 22, 432-438.

27) Nouri, Z., Hajialyani, M., Izadi, Z., Bahramsoltani, R., Farzaei, M. H., & Abdollahi, M. (2020). Nanophytomedicines for the prevention of metabolic syndrome: A pharmacological and biopharmaceutical review. *Frontiers in Bioengineering and Biotechnology*, 425.

28) Mans, K., & Aburjai, T. (2019). Accessing the hypoglycemic effects of seed extract from celery (*Apium graveolens*) in alloxan-induced diabetic rats. *Journal of Pharmaceutical Research International*, 26(6), 1-10.

29) Nelson, D. L., Lehninger, A. L., & Cox, M. M. (2008). *Lehninger principles of biochemistry*. Macmillan.

30) Salmenniemi, U., Ruotsalainen, E., Pihlajamäki, J., Vauhkonen, I., Kainulainen, S., Punnonen, K., … & Laakso, M. (2004). Multiple abnormalities in glucose and energy metabolism and coordinated changes in levels of adiponectin, cytokines, and adhesion molecules in subjects with metabolic syndrome. *Circulation*, 110(25), 3842-3848.

31) Achi, N. K., Ohaeri, O. C., Ijeh, I. I., & Eleazu, C. (2017). Modulation of the lipid profile and insulin levels of streptozotocin-induced diabetic rats by ethanol extract of *Cnidoscolus aconitifolius* leaves and some fractions: Effect on the oral glucose tolerance of normoglycemic rats. *Biomedicine & Pharmacotherapy*, 86, 562-569.

32) Lee, J. H., Gulumian, M., Faustman, E. M., Workman, T., Jeon, K., & Yu, I. J. (2018). Blood biochemical and hematological study after subacute intravenous injection of gold and silver nanoparticles and coadministered gold and silver nanoparticles of similar sizes. *BioMed Research International*, 2018.

33) Gargouri, M., Magné, C., & El Feki, A. (2016). Hyperglycemia, oxidative stress, liver damage, and dysfunction in alloxan-induced diabetic rats are prevented by Spirulina supplementation. *Nutrition Research*, 36(11), 1255-1268.

34) Rezaiyan, M., Sasani, N., Kazemi, A., Mohsenpour, M. A., Babajafari, S., Mazloomi, S. M., … & Zareifard, A. (2023). The effect of spirulina sauce on glycemic index, lipid profile, and oxidative stress in type 2 diabetic patients: A randomized double-blind clinical trial. *Food Science & Nutrition*, 11(9), 5199-5208.

35) Rostami, H. A. A., Marjani, A., Mojerloo, M., Rahimi, B., & Marjani, M. (2022). Effect of spirulina on lipid profile, glucose, and malondialdehyde levels in type 2 diabetic patients. *Brazilian Journal of Pharmaceutical Sciences*, 58.

36) Simon, J. P., Baskaran, U. L., Shallauddin, K. B., Ramalingam, G., & Evan Prince, S. (2018). Evidence of antidiabetic activity of *Spirulina fusiformis* against streptozotocin-induced diabetic Wistar albino rats. *3 Biotech*, 8, 1-12.

37) Rahnama, I., Arabi, S. M., Chambari, M., Bahrami, L. S., Hadi, V., Mirghazanfari, S. M., … & Sahebkar, A. (2023). The effect of Spirulina supplementation on lipid profile: GRADE-assessed systematic review and dose-response meta-analysis of data from randomized controlled trials. *Pharmacological Research*, 106802.

38) Kaur, S., & Singhal, B. (2012). When nano meets stem: The impact of nanotechnology in stem cell biology. *Journal of Bioscience and Bioengineering*, 113(1), 1-4.

39) Al-Dujaili, A. N. G., & Al-shemeri, M. K. (2016). Effect of silver nanoparticles and rosuvastatin on lipid profile in rats induced by high-fat diet. *Research Journal of Pharmaceutical Biological and Chemical Sciences*, 7(3), 1031-1037.

40) Ul Haq, M. N., Shah, G. M., Menaa, F., Khan, R. A., Althobaiti, N. A., Albalawi, A. E., & Alkreathy, H. M. (2022). Green silver nanoparticles synthesized from *Taverniera couneifolia* elicit effective anti-diabetic effects in alloxan-induced diabetic Wistar rats. *Nanomaterials*, 12(7), 1035.

41) Shannahan, J. H., Sowrirajan, H., Persaud, I., Podila, R., & Brown, J. M. (2015). Impact of silver and iron nanoparticle exposure on cholesterol uptake by macrophages. *Journal of Nanomaterials*, 2015, 1-1.

42) Ninan, N., Goswami, N., & Vasilev, K. (2020). The impact of engineered silver nanomaterials on the immune system. *Nanomaterials*, 10(5), 967.

43) Aissaoui, O., Amiali, M., Bouzid, N., Belkacemi, K., & Bitam, A. (2017). Effect of *Spirulina platensis* ingestion on abnormal biochemical and oxidative stress parameters in the pancreas and liver of alloxan-induced diabetic rats. *Pharmaceutical Biology*, 55(1), 1304-1312.