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Green-Synthesis of Iron Oxide Nanoparticles Via *Spirulina Platensis Algae* as a treatment for Iron Deficiency Anemia in rats

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The innovative aspect of this work is using green synthesis to determine the long-term effects of Iron Oxide Nanoparticles, which were discovered to alleviate anemia in prior studies.

Abstract

Anemia due to iron deficiency (IDA) is a global health issue that affects people of all ages. Green synthesis has become a dependable, sustainably sourced, inexpensive, and eco-friendly method for the synthesis of many metal oxide nanomaterials. *Spirulina Platensis algae* powder was used in this work to synthesize iron oxide (Fe_4O_3) nanoparticles (IONPs). The particles were examined, by transmission electron microscope (TEM), and X-ray diffraction (XRD). The resulting particles were spherical with an average diameter of 44 nm, according to the TEM data. The IONPs in the mixture were determined by using a magnetic Stirrer (MS). The long-term effects of IONP and their efficacy as an iron supplement for IDA treatment via biological experiment were evaluated. Thirty-six albino female adult rats were used as three sets (12 rats/each), the normal, the anemic, and the treatment group by IONPs till Hb at a normal level. Then 6 rats after treatment fed a normal diet for 2 weeks to estimate the effect of IONPs after stopped intake. Complete Blood Counts (CBC), Hb, serum Iron (Fe), and total iron binding capacity (TIBC) were determined; each transferrin (TF) and Transferrin saturation (TSAT)% were calculated. The blood markers revealed anemia in the anemic group. IONPs significantly ($P \leq 0.05$) improved each blood parameter for the anemia treatment compared to the control and anemic group. The therapy with a normal diet after IONPs did not significantly alter the blood indications. It has been concluded that the IONP had remediation of anemia with no long-term impacts.

Keywords: Green Synthesis, IONPs, Anemia, IDA, Spirulina

1. Introduction:

NUTRITIONAL ANEMIA may be defined as a reduction in hemoglobin concentration. Iron deficiency (ID) is the most prevalent cause, resulting in microcytic and hypochromic red cells in the peripheral smear [1]. Anemia may have multiple causes, According to WHO [2], inadequate iron (Fe) consumption causes 50% of instances of anemia.

Bacteria, seaweed, yeast, alga, and fungi are now being used to synthesize NPs in a biocompatible manner employing green nanotechnology. Due to their low cost and high manufacturing yield [3].

In the area of materials science, green synthesis has emerged as a dependable, long-lasting, and environmentally friendly method for the synthesis of several nanomaterials, including metal oxides, hybrids, and bio-inspired materials [4]. Thus, it is believed that a green synthesis is a crucial tool for minimizing the negative effects of nanoparticles that are frequently utilized in labs and manufacturing via traditional production methods. Biosynthetic processes are referred to as intracellular and extracellular synthesis based on where the nanoparticles formed from them occur [5]. The internal mechanism entails the electrostatic binding of metal ions or metal oxide ions to the microorganism's cell wall, which allows the ions to permeate into the cell and interact with certain enzymes to create IONPs [6]. The extracellular process involves the enzymatic reduction of iron ions to produce small-size distribution and evenly distributed NPs with genes, peptides, or proteins that serve as reducing agents, which in turn stabilizes and prevents IONP aggregation [7].

Microalgae, a group of primitive microscopic plants, have several benefits over larger plants when it comes to acting as cell factories for the manufacturing of nanoparticles. Microalgae and macroalgae are the two broad classifications for all algae [8]. Algae are regarded as the first microscopic plants and have few advantages over larger plants in terms of growth rate, food needs, and the production of nanoparticles [9]. As a result, scientists are very interested in employing algae to produce inorganic nanoparticles. There are three basic ways that algae are used to make nanoparticles. There are two other well-liked methods besides directly synthesizing nanoparticles from living algae cells: after the lysis of algal cells and extraction utilizing various downstream process techniques including centrifugation and filtration [10], nanoparticles are collected from the supernatants of different procedures.

During the synthesis of NPs, these natural materials are utilized as reducing, capping, and stabilizers since they include a variety of bioactive substances [11]. Amino acids, alkaloids, sugars, polyphenols, steroids, saponins, flavonoids, and terpenoids are a few of these ingredients. Specific microalgae species are widely employed because of their particular

bioactive components. the little blue-green algae called cyanobacterium, a filamentous prokaryote. One of the various types of microalgae that are grown and sold globally is *Spirulina platensis*. They can grow photographically, heterotrophically, or mixotrophically in a variety of settings, including freshwater, marine, and pond water with difficult conditions such as low light conditions, the presence of organic materials, and pollution [12]. They made it very simple to use in a range of industries, including pharmaceutical applications, the biofuel business, and wastewater cleanup, based on these assumptions and taking into account the benefits of *Spirulina platensis* [13].

Researchers in this sector also need to investigate the kinds of nanoparticles, the best sizes for them, and their ability to function as nanoscale biosorbents. Iron oxides are mostly employed due to their low cost and important roles in biological and geological processes. Because it doesn't require hazardous chemicals, the green synthesis of iron oxide nanoparticles using plant resources has various advantages, including low cost, eco-friendliness, and compatibility with a variety of applications [14].

The primary objective of this study was to examine the effectiveness of long-term impacts of iron oxide nanoparticles produced through the utilization of *Spirulina algae* powder as a sustainable synthesis technique. Also, this research pursued to evaluate these nanoparticles' potential as an iron supplement for treating iron deficiency anemia (IDA) through biological experimentation. Moreover, the researchers intend to ascertain whether the nanoparticles exhibit a lasting impact on the parameters associated with IDA.

2. Materials and Methods

2.1. Materials:

Spirulina palatensis algae were obtained from the Agriculture Research Center, Giza, Egypt.

Chemicals such as Casein ">85% protein", maize starch, DL-methionine, choline chloride, vitamins, and other necessary chemicals were bought from Morgan Com. for Chemicals in Cairo, Egypt.

Kits were bought from Bio-diagnostics Company in Cairo, Egypt

Thirty-six female Sprague Dawley rats (initial body weight 120±10 g) were obtained from the animal colony, Helwan Farm, Vaccine and Immunity Organization, Ministry of Health and Population- Helwan, Cairo Egypt

2.2 Methods

Prepare Iron Nanoparticles by *Spirulina palatensis* algae according to Shalaby et al., [15] as a following:

2.2.1 Preparation of Spirulina platensis micro-algal supernatant

Spirulina Micro-algal Supernatant Preparation Microalgal Powder (MALGP) of *Spirulina* was firstly splashed with normal tap water and then rinsed three times with deionized (DI) water to eliminate observed impure particles. The 12 g microalgal Powder of *Spirulina* has dissolved in 120 ml of deionized water in a 500 ml round-bottomed flask by stirring continuously using a magnetic stirrer (~ 150 rpm) for 1 h at 75° C. The homogenized solution was allowed to cool down naturally at 25 °C, the MAGLP was strained through a filter paper (Whatman paper of diameter 45 mm) and the clear supernatant solution was transferred in polypropylene tubes and set aside for the synthesis of *Spirulina* iron oxide nanoparticles (S-IONPs).

2.2.2 Spirulina platensis iron oxide nanoparticles (SP-IONPs) preparation

A typical approach involved dispersing 0.6 M of iron (III) of $FeCl_3.6H_2O$ in 95 mL of DI water and stirring it at a speed of about 200 rpm for one hour to guarantee complete dissolution. The iron (III) suspension was then gently added to 95 mL of MALGP supernatant at a volume ratio of 1:1 between the MALGP supernatant and Fe (III). The mixture's color abruptly changed from yellow to a deep brown, indicating that SP-IONPs had been successfully synthesized. The related solution was stirred for a further two hours, and the resulting homogeneous solution was placed in a hot air oven set at 75°C for 24 hours (fig. 1). Using a neodymium magnet, the resulting dark SP-IONPs (solid) were magnetically removed from the solution, washed four times with DI water, and then dehydrated at 70°C for six hours before being characterized.

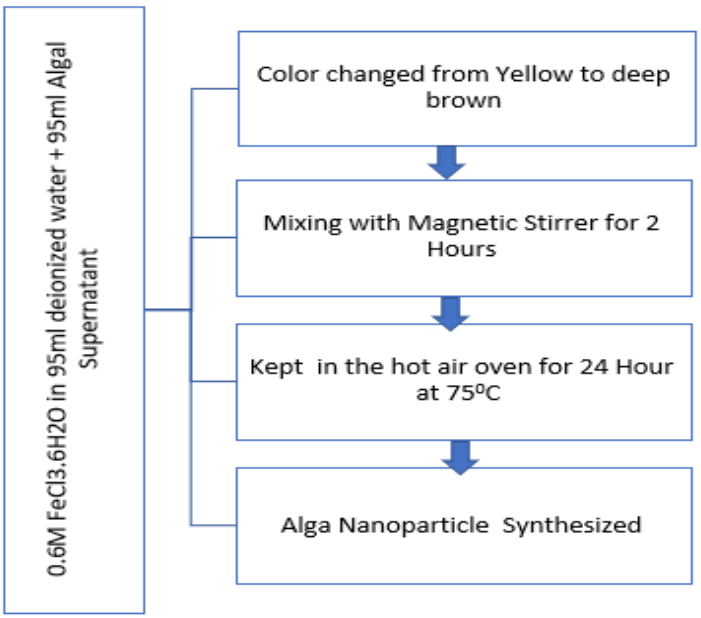

Fig. 1 Flow diagram showing the basic procedure for the synthesis of S-IONP

Structural characterizations as particle size and morphological features of nanoparticles by the lab of science faculty, Cairo University.

Several analytical tools have been employed to investigate the prepared nanomaterials to track the potential changes that may occur. However, their morphologies were scanned via the actual wavelength in nm of the produced IONPs was determined from UV-vis spectra, the high-resolution transmission electron microscope (HR-TEM,) (JEOL TEM-2100, Japan) with an accelerating voltage of 200 kV and magnification of 25 X. X-ray diffraction analysis (XRD) (Benchtop Minifex- II, Rigaku) is to determine the crystallographic structure of a material. Their sizes were studied by a dynamic light scattering device (DLS) manufactured by NIcomp company, USA model of Z300s. Fourier-transform infrared (FTIR) spectroscopy analysis of NPs by the *Spirulina algal* extract solution was utilized to determine the efficient groups of the active components. The FTIR spectrophotometer model used was the Perkin Elmer spectrum, a standard optical system with KBr powder, 4000–500 cm−1 is the spectral range used for the collection of data, at the highest resolution of 0.5 cm−1. Identification of the spectral absorption bands was made according to previously published information.

2.2.3 SP-IONP was measured using the magnetic stirrer method

Before using a magnetic stirrer (MS), which was weighed, a solution (5 g of SP-IONP was dissolved in 50 ml of deionized water), was produced. The solution was then shaken automatically for 10 minutes with MS. The magnetic stirrer was then weighted after that. Iron was then calculated [16].

$$\text{IONP weight / g} = \text{Wt. MS after} - \text{Wt. MS before}$$

2.2.4 Biological design

The National Hepatology and Tropical Medicine Research Institute's (NHTMRI) research ethics committee gave its approval before the study was carried out by Protocol No. A10/2022, which is the Guide for the Care and Use of Laboratory Animals.

Thirty-six female Sprague Dawley rats were kept separately in clean, well-ventilated cages for one week while being provided a basal/normal diet as per Reeves et al. [17] with some modifications and tap water available at all times. The rats were separated into groups as shown in (Fig. 2) after this time:

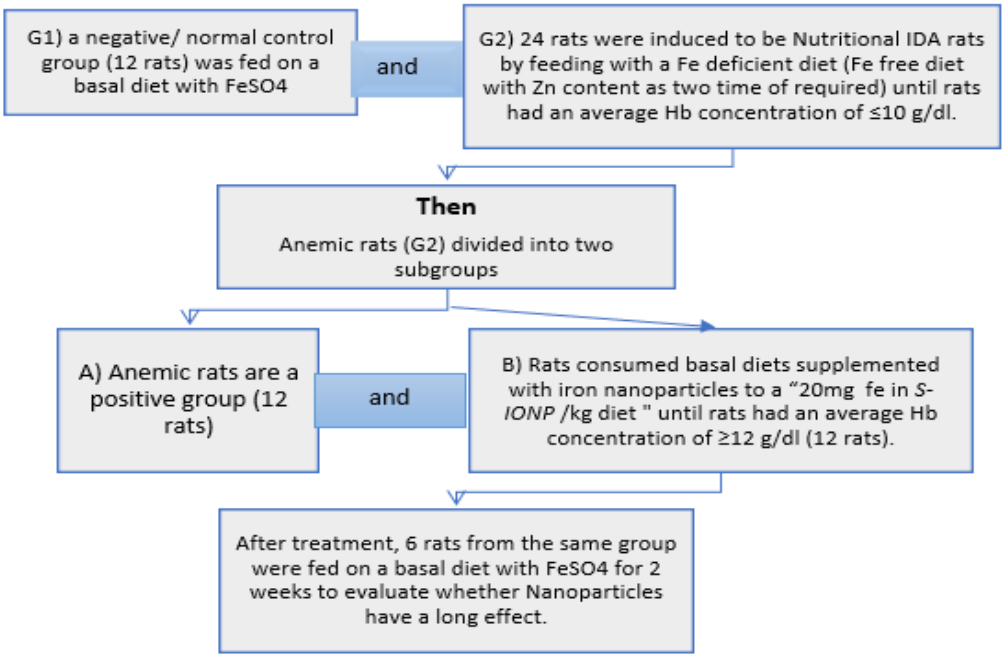

Fig. 2 Flow diagram showing rats' experimental design

During the experiment, hemoglobin was determined every week in whole blood by Rice [18]. When rats are treated and have an average Hb level of ≥12g/dl, blood is drawn from the orbital venous plexus under light anesthesia through heparin-coated capillaries into an EDTA-coated capillary tube for immediate complete Blood Count (CBC) analysis by using a hematology Analyzer, PIAG on Lt-D-cell 60 in the lab of National Nutritional Institute. The serum was estimated as iron (Fe) and total iron binding capacity (TIBC) by using a spectrometer on the report of Ginder, [19] and Piccardi et al., [20]. Calculated transferrin (TF) and Transferrin saturation (TSAT)% according to Elsayed et al., [21] as equations: transferrin =TIBC /1.4 µg/l and Transferrin saturation (TSAT)% according to Eleftheriadis et al., [22] TSAT% = Fe/ TIBC × 100 or TSAT%= Fe/ TF) × 70.9.

2.2.5 Histopathological examination:

The liver and spleen were taken out of the body and rinsed with a sodium chloride solution con. 0.9%, dried, and weighed. Before being preserved in paraffin with a 10% neutral buffering formaldehyde solution with a pH of 7.5, a portion of each organ was cleaned with xylol. For histological investigation, a section between 4 and 5 ml thick was taken out and stained with hematoxylin and eosin (H&E) [23].

2.2.6 Statistical analysis

The statistical analysis was conducted using the SPSS 19 software. Statistical methods were employed to indicate the disparity between means with a significance level of $P < 0.05$. The data was presented using the mean

and standard deviation (SD). The statistical analysis employed in this study was conducted using a one-way analysis of variance (ANOVA) and subsequent post-hoc testing (LSD) to identify significant differences, as outlined by Snedecor and Cochran [24].

3. Results

Figure (3) demonstrates using scanning spectroscopy, the peak absorption wavelength of the S-IONPs solution was found to be between 280 and 380 nm, with a large, sharp maximum absorption band at 300 nm. A transmission electron microscope (TEM) image of SP-IONPs produced by *Spirulina algae* in combination with a zoom TEM image of the spherical shape. Figure 3's DLS curve illustrates the size of IONPs which are roughly 44 nm in size and are in line with TEM results. The actual wavelength in nm of the produced IONPs was determined from UV-vis spectra and compared to that of alga.

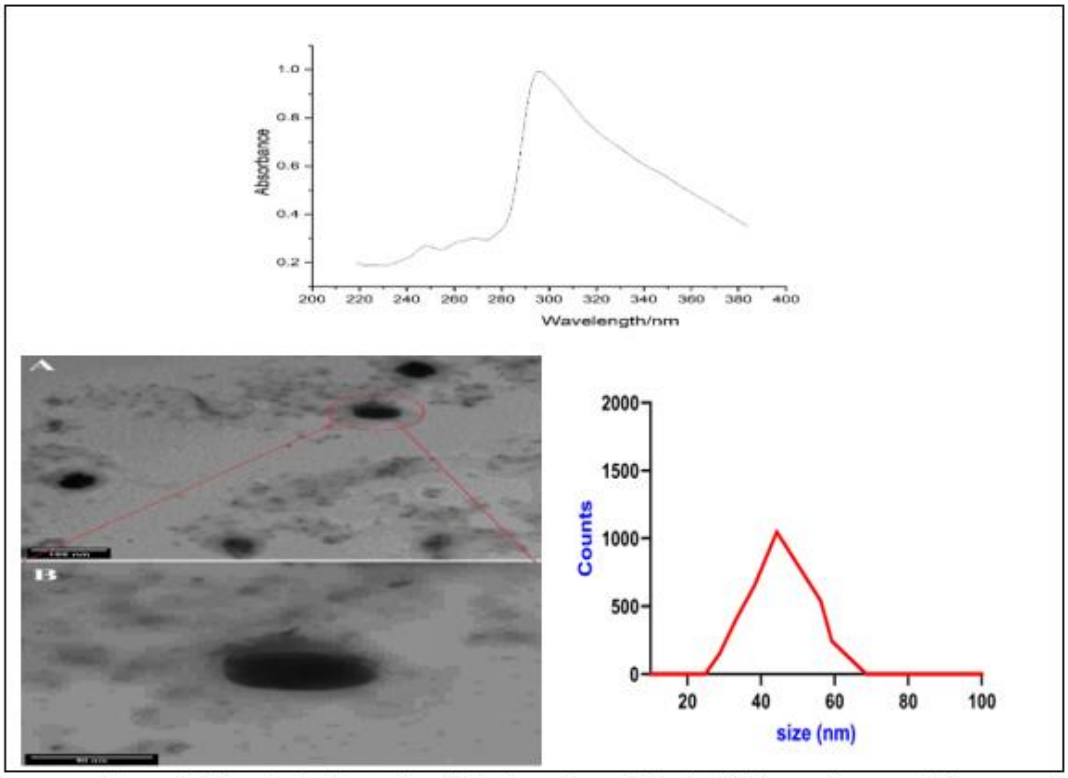

Figure 3: Characterizations of particle size and morphological features of nanoparticles

Figure 4 indicated the hemoglobin levels (g/dl) of rats without a normal control group at the beginning and weekly until the rats' average hemoglobin concentration was less than 10 g/dl (anemic rats), which induced nutritional iron deficiency in anemic rats feeding with a Fe-deficient diet (A Fe-free diet with Zn content as two times from required).

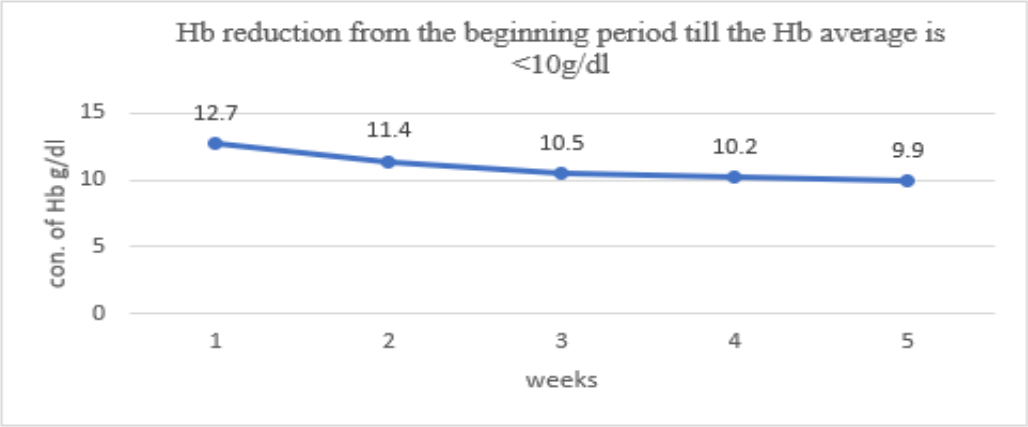

Fig. 4 Flow diagram showing Hb reduction of the anemic group (G2) during a period (4 weeks) or till the Hb average is less than 10g/dl

The data presented in Table 1 describes the CBC of rats in various groups in comparison to the control group. The anemic group had the lowest and most significant hemoglobin (Hb), hematocrit (HCT), red blood cell (RBC), and white blood cell (WBC) levels from other groups. However, no significant differences between groups 1, 3, and 4. The anemic group's Hb, HCT, and RBC concentrations changed by - 32.8, -35.1, and -31.3 percent compared to the normal group. However, the anemic group's WBC was higher than that of the other groups with a significant difference (P=0.011). Group 3 was treated with SP-IONP and in Group 4, the percent change was higher than normal and anemic rats for their parameters. Platelet count (PLT) tests revealed significant differences in all groups (P< 0.001), with the SP-IONP group displaying the highest value. In addition, the results indicate that G4, which underwent SP-IONP treatment before being placed on a normal diet, exhibited somewhat lower levels of all parameters compared to G3, but these differences were not statistically significant. The obtained results provide evidence that the *Spirulina* iron oxide nanoparticles (SP-IONP) did not have a significant long-term effect.

Table 1: Mean ± SD. Of Complete Blood Count for rats at the end of Trail

Groups / Parameters	G1	G2	G3	G4	*P* value Between groups
Hb g/dl	12.5±0.4[a]	8.4±1.2[b]	13.4±1.0[a]	12.7±0.3[a]	0.004
P value within groups	G1	0.004	0.470	0.898	
		G2	<0.001	0.003	
			G3	0.551	
%change vs G1	--	- 32.8	7.2	1.6	
%change vs G2	48.8	--	59.5	51.0	
HCT %	37.3±1.1[a]	24.2±3.2[b]	40.0±2.6[a]	37.5±1.0[a]	0.001
P value within groups	G1	0.004	0.16	0.504	
		G2	<0.001	0.001	

Groups / Parameters	G1	G2	G3	G4	*P* value Between groups
			G3	0.434	
%change vs G1	--	- 35.1	7.3	0.54	
%change vs G2	51.1	--	65.3	55.0	
RBCs 10^3/µl	6.7±0.3[a]	4.6±0.8[b]	7.4±0.4[a]	7.4±0.35[a]	0.006
P value within groups	G1	0.013	0.324	0.375	
		G2	0.002	0.002	
			G3	0.918	
%change vs G1	--	- 31.3	10.4	10.4	
%change vs G2	45.6	--	60.9	60.9	
WBC 10^3/µl	6.3±0.7[a]	18.0±4.3[b]	11.1±0.4[a]	7.4±0.4[a]	0.011
P value within groups	G1	0.003	0.151	0.743	
		G2	0.0.046	0.005	
			G3	0.259	
%change vs G1	--	185.7	76.2	17.5	
%change vs G2	- 65.0	--	- 38.3	- 58.9	
PLT 10^3/µl	158.0 ±4.9[d]	641.5±17.8[c]	1096.0±30.7[a]	779.0±25.1[b]	< 0.001
P value within groups	G1	< 0.001	< 0.001	< 0.001	
		G2	< 0.001	< 0.001	
			G3	< 0.001	
%change vs G1	--	306.0	593.7	393.0	
%change vs G2	- 73.4	--	70.8	21.4	

G1 (normal rats); G2 (anemic rats); G3 (rats fed S-IONP); G4 (rats after treatment and fed a normal diet)
Means subscribed in the same row with completely different letters is significantly different at p<0.05.

The finding in Table 2 revealed the overall serum iron (Fe), total iron-binding capacity (TIBC), Serum transferrin (TF), and transferrin saturation (TSAT) levels for all groups of experimental animals. When compared to other groups, the iron level of the anemic group (G2) was significantly the lowest. Iron levels were the highest in the normal group. Groups 3 and 4 had different iron values, but they were not found to be significantly different. The anemic group had the highest TIBC value, however, with significant differences from all other categories. The anemic group dramatically exceeded the difference from the other groups, with a percent change of 114.6 % when compared to the normal group. The same table showed that G4, which had been given a normal diet after receiving SP-IONP treatment, had levels of all parameters lower than G3 with non-significant differences. The TF and TSAT levels in the anemic group were lower than other groups and there was significant variation between the groups as well. Two groups, 3,4, with percentage changes of -7.8; -20.8, and -44.0; -53.2 respectively, showed lower TF and TAST levels than the normal group. These findings support the idea that the S-IONP did not have an ongoing effect.

Table 2: Serum Fe, TIBC, ST, and TSAT of rats in the End experimental

Groups / Parameters	G1	G2	G3	G4	*P* value Between groups
Serum Iron mg/l	26.9±0.8[a]	8.1±0.8[c]	24.8±2.1[ab]	21.3±1.1[b]	<0.001
P value within groups	G1	<0.001	0.163	0.024	
		G2	<0.001	<0.001	
			G3	0.250	
%change vs G1	--	- 69.9	- 7.8	- 20.8	
%change vs G2	232.0	--	206.2	163.0	
Serum TIBC mg/l	94.4±5.4[c]	202.6±1.3[a]	147.5±1.2[b]	155.4.0±3.1[b]	<0.001
P value within groups	G1	<0.001	<0.001	<0.001	
		G2	<0.001	<0.001	
			G3	0.126	
%change vs G1	--	114.6	56.3	64.6	
%change vs G2	-53.4	--	-27.2	-23.3	
Serum Transferrin	19.2±0.5[a]	5.8±0.6[c]	17.7±1.5[b]	15.2±0.8[b]	<0.001
P value within groups	G1	<0.001	0.275	0.044	
		G2	<0.001	<0.001	
			G3	0.261	
%change vs G1	--	-69.8	-7.8	-20.8	
%change vs G2	231.0	--	205.2	162.1	
Transferrin saturation (TSAT)%	29.3±1.2[a]	4.0±0.4[c]	16.4±1.9[b]	13.7±0.8[b]	<0.001
P value within groups	G1	<0.001	<0.001	<0.001	
		G2	<0.001	<0.001	
			G3	0.148	
%change vs G1	--	-86.3	- 44.0	- 53.2	
%change vs G2	632.5	--	310.0	242.5	

G1 (normal rats); G2 (anemic rats); G3 (rats fed S-IONP); G4 (rats after treatment and fed a normal diet)
Means subscribed in the same row with completely different letters is significantly different at p<0.05.

The outcomes reported in Table 3 demonstrated the organs' relative weights (liver and spleen). The findings showed no significant difference between all groups in both organs, which were different in value. Rat liver percent change in groups 2, 3, and 4 compared to normal control was (-15%), (-2.6%), and (-7.7%) respectively. Additionally, the percentage changes in spleen weight relative to normal rats were (-17, -5.9, and -8.8). Group 4 had lower levels in the two organs' relative weight than group 3 and group which fed the normal diet. These results support the hypothesis that the S-IONP had no lasting effects.

Liver Examination:

Samples were stained using H&E stain and examined using a light microscope at a magnification power of 400X. Hereafter are full details for the examined slides. The anemic group showed slight congestion of hepatic sinusoids and cytoplasmic vacuolization of centrilobular hepatocytes

Table 3: The relative weight of the liver and spleen in all groups

Groups / Parameters	G1	G2	G3	G4	*P* value Between groups
The relative weight of the Liver	3.9±0.2[a]	3.3±0.1[a]	3.8±0.1[a]	3.6±0.3[a]	0.158
P value within groups	G1	0.058	0.932	0.357	
		G2	0.068	0.279	
			G3	0.401	
%change vs G1	--	-15.4	-2.6	-7.7	
%change vs G2	18.2	--	15.2	9.1	
The relative weight of Spleen	0.34±0.03[a]	0.28±0.05[a]	0.32±0.02[a]	0.31±0.04[a]	0.824
P value within groups	G1	0.363	0.706	0.645	
		G2	0.578	0.645	
			G3	0.933	
%change vs G1	--	-17.6	-5.9	-8.8	
%change vs G2	21.4	--	14.3	10.7	

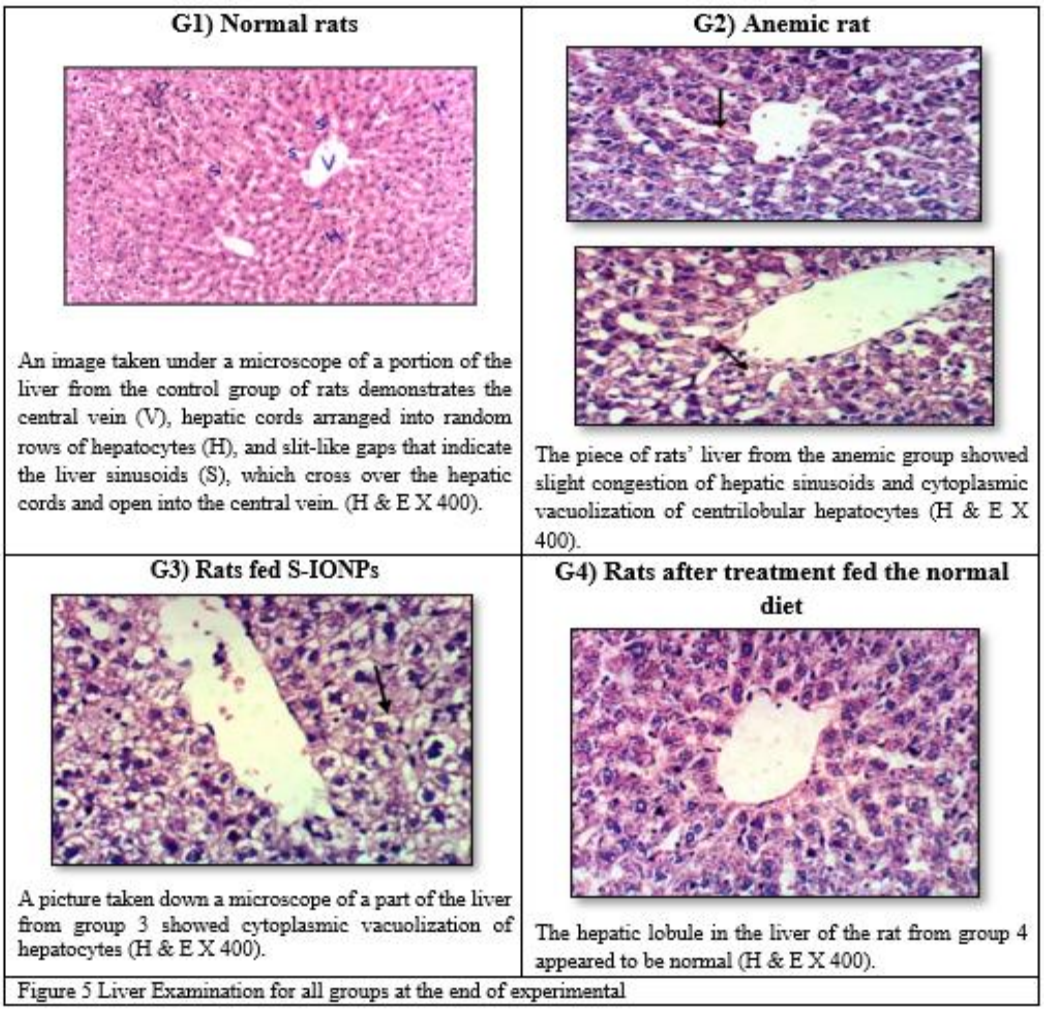

Figure 5 Liver Examination for all groups at the end of experimental

Spleen Examination

Samples were stained using H&E stain and examined using a light microscope at a magnification power of 400X. Hereafter are full details for the examined slides. Spleen Specimen of a rat from the anemic group (G2) shows slight lymphocytic necrosis, depletion, and thickening of the splenic capsule.

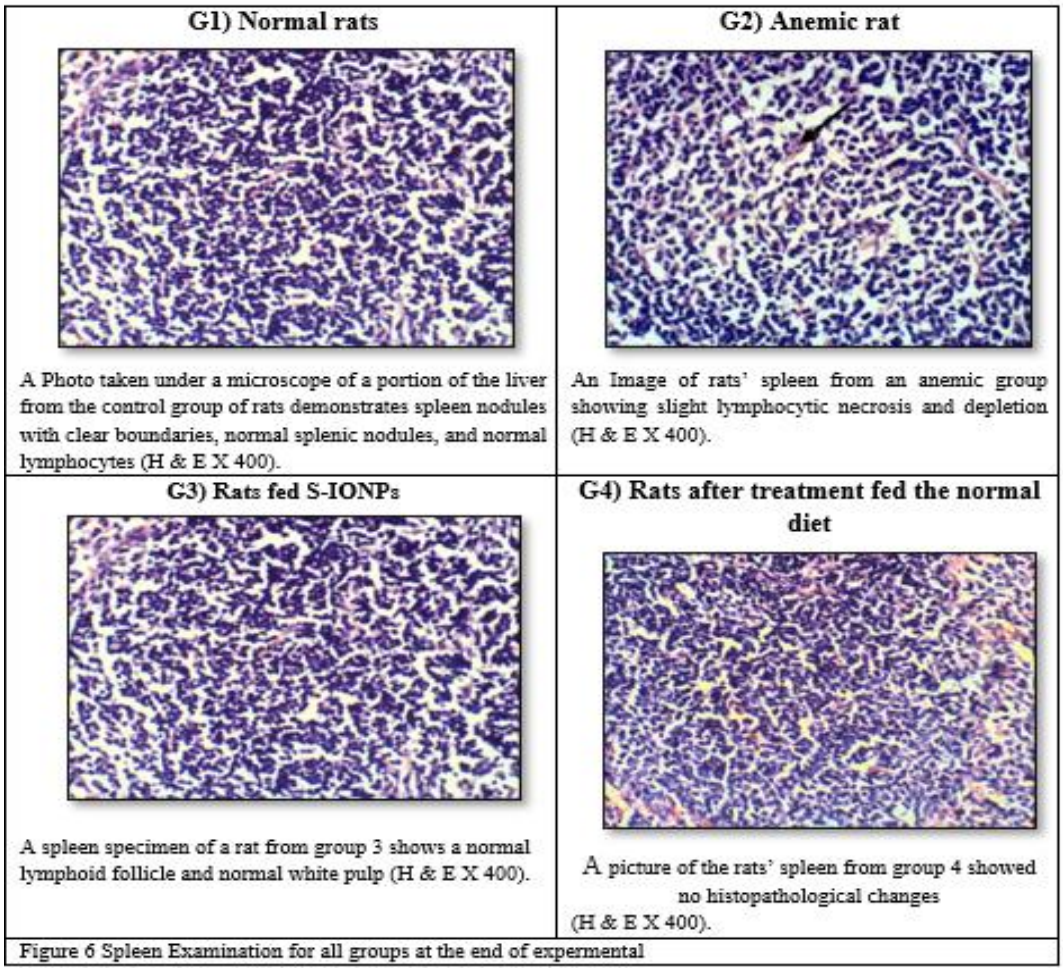

Figure 6 Spleen Examination for all groups at the end of expermental

4. Discussion

The stability, size, aggregation, and structure of the NPs have all been investigated using ultraviolet analysis [25]. When incident light rays interact with the conduction band electrons on the surface of the metal NPs, each metal oxide and metal NP has a unique absorbance wavelength, which is determined by the UV spectrum [26]. According to Aisida et al.,[27], the UV spectrum's 280 to 450 nm region corresponds to the particular absorbance band for IONPs Fig. 3 was 280-380nm may be a different spectrum system or existence *Spirulina*. According to Ibraheem et al., [28], the spectrum produced by FTIR spectroscopic analysis resembles a fingerprint with absorption peaks that match the wavelength of vibrations occurring within the confines of the NPs. Figure 3 displays the key functional groups that

were in charge of the reduction process during the synthesis of IONPs as determined by earlier research. One of the widely employed characterization instruments for determining the form, dimension, and morphology of IONPs is the transmission electron microscope (TEM) [29]. The obtained samples are dried using a mercury lamp to facilitate the simple passage of a monochromatic electron beam through the sample, which results in a picture on the viewing screen [30]. Numerous researchers have described the use of TEM in the morphological evaluation of IONPs synthesized, from various plant sources, which is consistent with the current findings shown in Fig. 3.

In this search, the administration of SP-IONPs led to significant improvements in every CBC parameter as compared to the control group for the treatment of anemia Table (1). Iron oxide nanoparticles' enhanced bioavailability and absorption may have been caused by their noticeably small size [31]. These iron oxide nanoparticle impacts on the CBC parameter lined up with findings from Shafie et al., [32]. Additionally, the group that was given an iron-free basal diet had anemia, as shown by the blood indicators. There was no noticeable distinction between the treatment with iron oxide nanoparticles or ferrous sulfate and the normal control in the blood parameters. The current finding was consistent with that of Hashem et al., [33], who noted a considerable rise in red blood cell indices in the iron oxide nanoparticles group as compared to the anemic group. This could be attributable to iron oxide nanoparticles' potency in the treatment of anemia without long effect.

Serum iron levels in the anemic group shown in Table 2 are lower than the usual 40–165 mg/L [34]. The fast changes in serum iron caused by factors like dietary intake and circadian rhythm make it difficult to determine iron status purely by serum iron testing [35]. Inflammation causes a reduction in iron status [35, 36]. According to study findings in Table 2, administering iron oxide nanoparticles to rats after causing anemia is a very successful way to treat the condition without having any remaining, effects. A considerable rise in serum iron and transferrin saturation compared to the anemia group provides proof of this. According to Criswell et al., [37], an increase in serum iron levels and transferrin saturation is a sign that anemia is being treated. It is crucial to consider the variation in serum iron concentrations when interpreting the results of the TSAT [38]. Total iron binding capacity significantly increased in the anemic group, which may be related to the liver's enhanced production of transferrin to make the most of the limited amount of iron that is there. The total iron binding capacity significantly decreased in the iron oxide nanoparticles group as compared to the control group, demonstrating the efficiency of these particles in treating anemia. The current finding agreed with those of Zariwala and others [39]; and Shafie

et al., [32]. TSAT or transferrin saturation offers details on the availability of iron. TSAT has good sensitivity and specificity [40], is frequently used in clinical practice, and is recommended in several guidelines and research [35, 41, 42]. It should be noted that the value of TSAT is subject to variation because serum iron and transferrin are both affected by the myriad of factors mentioned above [35]. TSAT should always be evaluated in the morning while fasting due to the influence of the circadian cycle and dietary intake of iron [38]. Studies have shown that TSAT fluctuates less than serum ferritin in inflammatory situations, suggesting that inflammation has less effect [38, 40, 43]. TSAT can therefore be used to identify ID in patients with chronic disorders [43, 44].

Based on the data in Table 3, there is no appreciable difference between the relative weights of the liver and spleen, which is consistent with the findings shown in Figures 5 and 6. The liver revealed adverse effects from IONPs, while the spleen didn't. These findings are consistent with those of Kulkarni et al. [45], who discovered that the histological analysis was significantly impacted by IONPs at the concentrations (10 mg/kg and 100 mg/kg). The liver's hepatocytes display nuclear degeneration. These detrimental cellular alterations did not occur in either the vehicle or control groups. Superparamagnetic iron oxide nanoparticles were tested on Sprague Dawley rats in other literature, and the results showed no changes in histopathology [46].

5. Conclusion:

The present study focuses on the eco-friendly synthesis, characterization, and long-term impacts of iron oxide nanoparticles (IONPs) derived from *Spirulina platensis algae* (SP). Based on the X-ray diffraction (XRD) patterns, it was determined that the nanoparticles generated exhibited a crystalline structure and were in a state of -Fe2O3. The iron content is verified through the utilization of magnetic starrier (MS) analysis. Hematological tests, biochemical analyses, histological investigations, and assessments of organ mass collectively support the utilization of iron oxide nanoparticles (IONPs) for the treatment of anemia. The rats' substantial improvement in anemia persisted for two weeks following the cessation of feedings with iron oxide nanoparticles (IONPs). Using SP alga in the protocol for green biosynthesis of IONPs offers numerous advantages compared to chemical synthesis. The plant species of SP alga exhibit a diverse range of bioactive compounds while offering sustainable resources that can be utilized for the large-scale manufacture of nanoparticles (NPs).

Conflict of interest

The authors of this article has indicated that they has no conflicts of interest to disclose.

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6. References:

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