

Role of Spirulina Loading Chitosan to Mitigate Phenyl Hydrazine Induce Hemolytic Anemia

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Abstract—Background: Hemolytic anemia, often experimentally induced using phenylhydrazine (PHZ), results in oxidative damage to red blood cells (RBCs), leading to reduced hematological parameters. Spirulina, a blue-green microalga rich in antioxidants and essential nutrients, has shown therapeutic potential in treating anemia. Enhancing its bioavailability through nano-encapsulation with chitosan may further improve its efficacy. **Methods:** Anemia was induced by phenylhydrazine intraperitoneal (20 mg/kg) for 2 following days. A total of 40 adult male albino rats were used in the present study, at the age of 2 months with body weight 165 ± 15 gm were separated randomly to four equal groups (10 rats for each) and treated as follows for 8 weeks: Control group (CNG): -in this group animals left without any treatment like negative control. PHZ-G: animals in this group were induced anemia and untreated as positive group. SPG: animals in this group were induced anemia and treated with daily dose 50mg/kg of spirulina pure given orally by stomach tube. SP-NPS: animals in this group were induced anemia and treated with daily dose 50mg/kg of CNP-S given orally by stomach tube. At the end of the experiment, all animals were sacrificed and blood samples (5ml) were collected directly from the heart by the cardiac puncture. **Results:** The chitosan-encapsulated Spirulina nanoparticles exhibited a mean particle size of 93.2 nm and a low polydispersity index (0.005), indicating high stability. Anemic rats showed significant reductions in hematological indices. Treatment with Spirulina, especially in nanoparticle form, significantly improved HCT and reduced MCV, MCH, and MCHC compared to the anemic group, indicating recovery from anemia. **Conclusion:** Spirulina-loaded chitosan nanoparticles demonstrated enhanced stability and therapeutic efficacy against PHZ-induced anemia, making them a promising strategy for improving hematological health through targeted antioxidant delivery.

Keywords— PHZ, Spirulina, Hemolytic anemia, chitosan, SEM.

I. INTRODUCTION

A cyanobacterium, or blue-green algae, well-known for its high nutritional value and potential health benefits in the spirulina. It is mostly composed of the species *Arthrospira platensis* and is grown in fresh and marine waters all over the world. Spirulina is a popular nutritional supplement because of its high protein content and abundance of vital elements, including vitamins, minerals, and antioxidants.^{[1][2]}

The nutritional contents: Proteins, vital amino acids, and vitamins B12, A, C, and E are all abundant in spirulina [3]. It has colors like phycobiliproteins and chlorophyll A, as well as important fatty acids like γ -linolenic acid. Among the minerals found are iron, calcium, magnesium, and zinc.^[3] Health human benefits : Spirulina is related with different health benefits, involving hypotensive, hypocholesterolemia, and seasonal allergies.^[3, 4]

^[2] It may help delay or treatment cases like obesity, cancer, oxidative stress, cardiovascular diseases diabetes, and anemia, It has been observed to have immune-stimulating effects and may offer protective benefits against chemotherapy toxicity.^[5] uses for therapy: illness prevention, according to data, spirulina may have hepatoprotective and antiviral activity that help prevent and treat a number of health problems .^{[6][7]}

In fact the most a common defect characterized by decrease hemoglobin levels or a lower red blood cell mass.^[8] It can result from several factors, including dietary deficiencies, especially the most prevalent kind, iron deficiency[8]. The disorder frequently goes undetected until routine testing finds it, but it can have serious health effects like poor cognitive development and higher maternal

mortality. types of anemia: iron deficiency anemia (IDA): Frequently related to dietary choices or blood loss, anemia is caused by insufficient iron intake or absorption. anemia of chronic disease: connected to chronic infections or inflammatory disorders that impair the synthesis of red blood cells. a plastic anemia: results from bone marrow failure, leading to decreased red blood cell production.

The incorporation of Spirulina into chitosan-based nanoparticles has shown promising results in enhancing the delivery and efficacy of bioactive compounds, particularly for antibacterial applications. This approach leverages the unique properties of both Spirulina and chitosan, leading to improved stability, bioavailability, and therapeutic effects. antibacterial properties: Spirulina platensis contains bioactive compounds such as saponins, tannins, and phenols, which exhibit antibacterial activity against pathogens like *Staphylococcus aureus* and *Pseudomonas aeruginosa*, Chitosan nanoparticles loaded with Spirulina extract demonstrated enhanced antibacterial activity compared to the extract alone, indicating that nanoencapsulation improves the uptake of bioactive compounds by bacterial cells.^[9] formulation and characterization, Various studies have utilized chitosan to coat lipid nanoparticles and nanoliposomes containing Spirulina, resulting in controlled release and improved stability of the encapsulated compounds^[10], The size and zeta potential of these nanoparticles can be optimized by adjusting the concentration of chitosan, which affects their dispersion and encapsulation efficiency.^{[11] [12]}, Clinical studies have appear that formulations utilizing nanoparticles can effectively treatment acne skin lesions, While the advantages of Spirulina-loaded chitosan are evident, challenges still in optimizing formulations for specific applications and ensuring

persistent therapeutic outcomes across diverse skin types and pathological conditions.

II. MATERIAL AND METHODS

Loading of Spirulina onto Chitosan Nanoparticles (SCNPs): Based on the methods described [13, 14] with [14, 15] slight modifications, 200 mg of spirulina powder was dissolved in 1 ml of distilled water. This solution was gradually added dropwise to 50 ml of a chitosan solution (4 mg/ml) under continuous stirring at 900 rpm for 30 minutes to facilitate the encapsulation of spirulina within the chitosan nanoparticles. The resulting mixture was then subjected to sonication for 5 minutes. Following sonication, stirring was resumed, and 10 ml of sodium tripolyphosphate (TPP) solution (0.25%) was added slowly in a 5:1 volume ratio to the chitosan-spirulina mixture. The solution was stirred for another 30 minutes to promote the adsorption of spirulina onto the chitosan nanoparticle surfaces. Afterward, the mixture was sonicated again for 5 minutes and subsequently filtered using filter paper to remove unbound particles. Finally, the filtrate was centrifuged at 10,000 rpm for 15 minutes. The resulting pellet, containing the spirulina-loaded chitosan nanoparticles, was collected while the supernatant was discarded.

III. MATERIALS AND METHODS

Animals

Sixty healthy male albino rats (*Rattus norvegicus*), 8 weeks old and weighing 165 ± 15 g, were sourced from the Animal House, College of Veterinary Medicine, Al-Qasim Green University. They were housed in standard plastic cages ($12 \times 15 \times 29$ cm) with weekly-changed wood shavings, and provided free access to standard pellets and water. Rats were acclimatized for two weeks before the study.

Induction of Anemia

Hemolytic anemia was induced in 30 rats by intraperitoneal (IP) injection of phenylhydrazine (PHZ) at 20 mg/kg for two consecutive days. Anemia was confirmed on day 4 based on clinical and hematological indicators.

Experimental Design

Rats were randomly assigned to four groups (n = 15 each): NCG (Normal Control Group): Untreated. PHZ Group (Positive Control): Received PHZ and distilled water orally. SP Group (Spirulina): Received PHZ, then treated with Spirulina (50 mg/kg/day, orally). SP-NPS Group (Nano-Formulated Spirulina): Received PHZ, then treated with CNP-SP (34.5 mg/kg/day, orally).

Sample Collection

At the end of the study, 5 ml blood was collected by cardiac puncture. One ml was placed in EDTA tubes for CBC, while 4 ml was transferred to gel tubes, clotted for 30 minutes, and centrifuged at $3000 \times g$ for 3 minutes. Serum was stored at -20°C for further analysis.

IV. RESULTS

Characterization of Nanoparticles

The particle size was measured by laser rays that penetrated the liquid containing the molecules of spirulina encapsulated within chitosan. Our data appear that the range particle size of spirulina after delivered by polymers was an average of 93.2nm as showed in fig (1). The Polymer dispersion index (PDI), also known as the Polydispersity Index, is a measure of the distribution of molecular mass of polymer. Data appear uniformity of nanoparticles, with perfect polydispersion index at 0.005 that refer good stability. Particle sizes range from ~40–100 nm, though aggregation may affect accuracy. Morphologically, the particles appear irregular, aggregated, and rough-surfaced, indicating potential chitosan–Spirulina interaction fig(2).

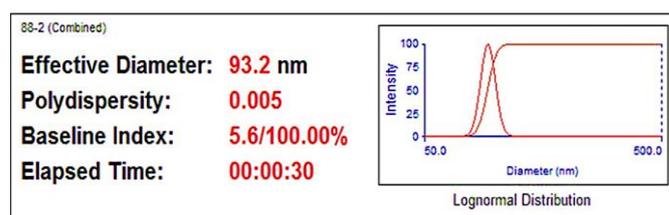


Fig (1): The effective diameters of spirulina loading chitosan nanoparticles showed 93.2nm with polydispersion index 0.005.

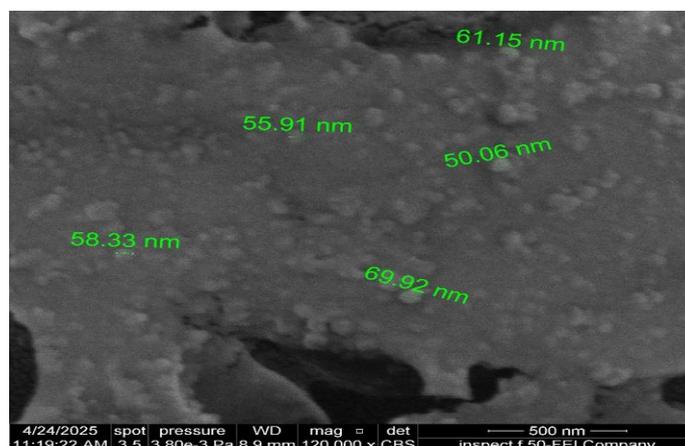


Fig (2): The effective diameters of spirulina loading chitosan nanoparticles showed 93.2nm with polydispersion index 0.005.

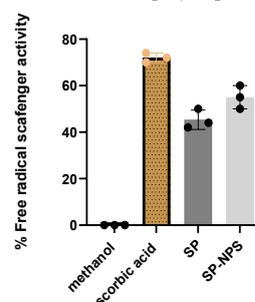


Fig (3) Role of spirulina and spirulina loading chitosan on % Free radical scavenger activity

Red Blood Corpuscles Parameters

The mean of (MCV, MCH, and MCHC) in anemic group in table showed a significant increase ($P \leq 0.05$) as compared with NCG, while treatment groups SPG and SP-NPS MCV,

MCH and MCHC recorded a significant decrease when compared with anemic group and negative control group, while HCT showed a significant ($p \leq 0.05$) increase in anemic group that treated with 50mg/kg of spirulina loading chitosan nanoparticles as compare with other groups.

Phenylhydrazine (PHZ) is a well-known chemical agent used to experimentally induce hemolytic anemia in animal models, particularly rats. Its mechanism of action primarily involves oxidative damage to red blood cells (RBCs).^[16, 17] Upon administration, phenylhydrazine generates reactive oxygen species (ROS) and free radicals, which cause peroxidation of the lipid membrane of RBCs. This oxidative stress compromises membrane integrity, leading to increased fragility and premature destruction of erythrocytes. Moreover, PHZ directly interacts with hemoglobin, forming methemoglobin and Heinz bodies—denatured hemoglobin aggregates that are toxic to RBCs. These abnormal forms trigger recognition and clearance by macrophages in the spleen and liver, thereby shortening the lifespan of erythrocytes and reducing circulating RBC counts.^[18] This process mimics the pathophysiology of hemolytic anemia seen in various clinical settings. In addition to direct hemolysis, phenylhydrazine suppresses erythropoiesis in the bone marrow, further aggravating anemia.^[19] The combined effect of increased destruction and decreased production of RBCs leads to a marked drop in hemoglobin concentration and hematocrit levels.

TABLE (1): Effect of spirulina and spirulina loading chitosan nanoparticles on RBCs indices of anemic male rats.

GROUPS	HCT (%)	MCV	MCH (pg)	MCHC(g/Dl)
CNG	39.27±0.954 b	51.24±0.422 a	66.69±5.505 c	132.1±1.657 a
PHZ-G	29.54±1.415 c	76.36±2.099 c	83.16±1.312 a	142.5±2.295 a
SPG	43±1.301 a	54.29±1.114 a	67.21±0.986 b	127.8±1.009 b
SP-NPS	41.91±1.072 a	50.67±0.273 a	64.27±1.315 b	120.7±1.474 c
LSD	3.92	4.40	15.8	5.6

- The value represent mean ± SE
- N=10 for each group.
- Different small letters indicated significant ($p \leq 0.05$) among groups.
- CNG: Control Negative Group,
- PHZ-G: Anemic group injected with phz,
- SPG: An anemic group treated with spirulina pure group,
- SP-NPS: An anemic group treated with spirulina loading chitosan nanoparticles.

Spirulina, a blue-green microalga, is a rich source of biologically active compounds that contribute significantly to its therapeutic effects in managing anemia [20]. Among the key bioactive components are phycocyanin, iron, essential amino acids, vitamins (particularly B12 and folic acid), and antioxidants such as carotenoids and tocopherols.^[3, 21] Phycocyanin, the major pigment-protein complex in Spirulina, exhibits strong antioxidant and anti-inflammatory activities,

which help mitigate oxidative damage to red blood cells (RBCs) commonly seen in hemolytic anemia. Furthermore, Spirulina's high iron content, in a highly bioavailable form, supports erythropoiesis and replenishes iron stores necessary for hemoglobin synthesis.^[13]

In addition to its nutritional and antioxidant value, Spirulina's role is enhanced when its bioactive components are encapsulated with chitosan nanoparticles. Chitosan, a naturally occurring biopolymer made from chitin, has regulated release, biocompatibility, and mucoadhesion Yamgar and Dhamak [22]. When used as a delivery system, chitosan protects the sensitive compounds in Spirulina from degradation, improves gastrointestinal absorption, and prolongs their bioavailability. This nano-formulation increases the therapeutic efficiency of Spirulina by ensuring a sustained release of active ingredients, thereby promoting continuous stimulation of erythropoiesis and improving hematological parameters.

Moreover, the synergistic effect of Spirulina's antioxidant molecules and chitosan's protective delivery leads to enhanced protection of RBC membranes from oxidative stress induced by phenylhydrazine.^[21] This combination helps reduce hemolysis, improve red cell count, hemoglobin concentration, and overall hematological status in anemic conditions. The sustained release and improved absorption provided by chitosan nanoparticles make this an effective strategy in maximizing the hematinic potential of Spirulina.

V. CONCLUSION

The research findings indicated the feasibility of loading Spirulina onto chitosan polymers, resulting in high stability and an appropriately small particle size. Furthermore, both the Spirulina extract and its nanoparticle formulation demonstrated a significant role in improving anemia levels compared to the untreated group. This suggests their potential for broader application and achieving more realistic outcomes in future studies.

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