Original Article

Effect of Oral *Spirulina Platensis* Supplementation on Hematological Parameter in Long Evans Rats

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Abstract

Background: *Spirulina Platensis, a filamentous cyanobacterium, is a nutrient-rich microalga with high-quality protein, vitamins, minerals, and bioactive compounds. It has been associated with hematological and metabolic benefits, including enhanced erythropoiesis, antioxidant activity, and lipid modulation. This study evaluated the effects of oral Spirulina platensis supplementation on hematological parameters in Long-Evans rats.*
Materials and Methods: *Forty-two Long-Evans rats of both sexes were randomly assigned to two groups (n=21 each): control (normal saline) and Spirulina Platensis supplementation (400 mg/kg body weight) for 28 days. Rats were housed under standard laboratory conditions and acclimatized for seven days prior to the experiment. Blood samples were collected via the retro-orbital plexus 24 hours after the last administration. Hematological parameters (RBC, HGB, WBC, MCV, MCH, MCHC, PLT) were assessed using an automated analyzer. Data was analyzed using independent samples t-test, with $p<0.05$ considered statistically significant.*
Results: *Supplementation with Spirulina platensis (400 mg) significantly improved hematological parameters in rats compared to controls. WBC, RBC, and HGB increased significantly (WBC: 21.28 ± 4.30 vs 24.59 ± 2.95, p=0.007; RBC: 6.79 ± 0.87 vs 8.93 ± 0.89, p < 0.001; HGB: 14.39 ± 1.64 vs 20.69 ± 2.79, p <0.001). Significant increases were also observed in MCV, MCH, MCHC (all p < 0.001) and platelet counts (695.32 ± 137.64 vs 787.71 ± 143.67, p=0.040), indicating enhanced red and white blood cell indices and platelet levels.*
Conclusion: *Oral supplementation of Spirulina platensis at a dose of 400 mg/kg body weight for 28 days significantly improved red blood cell indices in Long-Evans rats. Specifically, RBC count, hemoglobin concentration (HGB), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were markedly increased compared with the control group, indicating enhanced erythropoietic activity. These findings suggest that Spirulina platensis can positively influence hematological health and may serve as a functional dietary supplement to support red blood cell production and overall oxygen-carrying capacity in animals.*

Keywords: *Spirulina platensis*, Hematological parameters, Long-Evans rats, Dietary supplementation.
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Introduction

Spirulina is a microscopic, filamentous cyanobacterium (blue-green alga) with a long history of use as a human food supplement. Several species of Spirulina, including *Spirulina platensis*, *Spirulina maxima*, and *Spirulina fusiformis*, are recognized as the valuable sources of both macronutrients and micronutrients. These include high-quality protein, iron, gamma-linolenic fatty acid, vitamins-particularly vitamin B12-minerals, carotenoids, and phycocyanins. The safety of the Spirulina as a food supplement has been well established through toxicological studies[1]. Numerous experimental studies have demonstrated the broad therapeutic potential of Spirulina. It has been reported to prevent or inhibit cancer development in animal models[1,2]. Both in vitro and in vivo studies suggest that Spirulina possesses antiviral properties and exerts potent immunomodulatory effects by enhancing phagocytic activity and natural killer (NK) cell function[1-4]. Additionally, Spirulina and other cyanobacteria have been shown to stimulate the macrophages, NK cells, T cells, and B cells, as well as promote the production of the antibodies and the cytokines in animal models and human volunteers[1,5-9].

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Spirulina has also been associated with beneficial metabolic effects. Hypocholesterolemic activity has been reported in animal studies[3,6,7]. Anuradha and Vidhya[4] demonstrated its antihyperglycemic effects in clinical trials, suggesting a protective role against the development of atherosclerosis and the maintenance of euglycemia. Furthermore, Layam and Reddy reported that *Spirulina* exerted antidiabetic effects in streptozotocin (STZ; 45 mg/kg body weight)-induced diabetes in male albino Wistar rats[1]. *Spirulina* is considered the world's richest natural protein source and an important medicinal alga[5,8,9]. Among the various species, *Spirulina platensis* and *Spirulina maxima* are the most commonly used as nutritional supplements[6,7]. Due to its established safety, Spirulina is listed by the United States Food and Drug Administration as a "Generally Recognized as Safe" (GRAS) substance[7].

The microalga *Spirulina platensis* is also recognized as a single-cell protein containing nearly all essential nutrients and vitamins, along with remarkable pharmacological properties when compared with other commonly used plant sources[8,9]. Although it contains approximately 55-70% protein by dry weight and has lower levels of methionine, cystine, and lysine compared to animal-derived proteins, Spirulina remains superior to most plant-based protein sources, including legumes. It is also rich in polyunsaturated fatty acids (PUFAs), thiamine, riboflavin, niacin, vitamin B6 (pyridoxine), and vitamin B9 (folic acid)[8].

Recent evidence indicates that *Spirulina* consumption plays an important role in obesity control by modulating appetite regulation, reducing food absorption, preventing hepatic lipid accumulation, lowering oxidative stress, and inhibiting macrophage infiltration into visceral adipose tissue[10]. A systematic review and meta-analysis reported that *Spirulina* supplementation has a beneficial impact on fasting blood glucose levels and lipid profiles[11]. Its high protein and fiber content contributes to reduced glucose absorption and enhanced insulin secretion[12].

Moreover, phycocyanin, the blue pigment present in *Spirulina,* exhibits strong antioxidant properties and has been shown to lower blood pressure through adiponectin stimulation[13]. *Spirulina* has also demonstrated antiviral, antimutagenic, and cholesterol-lowering activities, largely attributed to its carotenoids, minerals, and antioxidant components[8,14].

Based on these diverse biological and pharmacological properties, the present study aimed to evaluate the effects of oral *Spirulina Platensis* supplementation on hematological parameters in Long-Evans rats.

Materials and Methods

Study design and Ethical approval: This experimental study was conducted in the Department of Physiology, Bangladesh Medical University (BMU), Dhaka, and the Department of Pharmacology from March 2024 to February 2025. The study protocol was reviewed and approved by the Institutional Review Board of BMU. All experimental procedures were carried out in accordance with internationally accepted guidelines for the care and use of laboratory animals.

Experimental animals and housing conditions: A total of 42 Long-Evans rats of both sexes, weighing 400 mg/kg were obtained from the Bangladesh University of Health Sciences, Dhaka, Bangladesh. The animals were housed in specially constructed plastic cages (45×30×15 cm^3), with three to four rats per cage, under a controlled 12-hour light and 12-hour dark cycle[15,16] in the pain laboratory of the Department of Physiology, BMU. The rats were acclimatized to laboratory conditions for seven days before the commencement of the experiment. All efforts were made to minimize animal suffering and to use the smallest number of animals necessary. Each animal was used only once and was sacrificed immediately after completion of the experimental procedures. The ambient room temperature was maintained at 27.5±0.5°C, corresponding to the thermoneutral zone for rodents[17]. Standard laboratory chow[18] and cooled boiled water were provided *ad libitum* throughout the study period. All experiments were conducted during the daytime (08:00-16:00 hours) to avoid circadian influences[19].

Blood sample collection and biochemical analysis: On day 29, 24 hours after the last administration of *Spirulina Platensis*, the rats were anesthetized using diethyl ether (Cairo Erba Reactifs-SDS). Approximately 2 mL of blood was collected from the retro-orbital plexus using separation gel coagulant tubes. The blood samples were centrifuged at 5000 rpm for 12 minutes. For complete blood count (CBC) analysis, blood samples were collected in EDTA tubes and analyzed using an automated hematology analyzer with MZ-05 reagents, including diluent, Lyse-1, Lyse-2, and cleaning solution (Cobas c111, Germany).

Statistical analysis: Data was examined for normality prior to statistical analysis. Data was presented as mean ± standard deviation (SD). Differences between the two groups were analyzed using an independent samples *t*-test. Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS), version 25. A *p*-value of

less than 0.05 was considered statistically significant.

Results

Table-I demonstrates the effects of oral *Spirulina platensis* supplementation (400 mg) on hematological parameters in Long-Evans rats. The total white blood cell (WBC) count did not differ significantly between the control and Spirulina-supplemented groups (p=0.548), indicating that Spirulina had no appreciable effect on leukocyte levels. In contrast, a significant increase in red blood cell (RBC) count was observed in the Spirulina-treated group compared to the control group (mean difference=2.14; p<0.001).

Similarly, hemoglobin (Hb) concentration was markedly higher in the Spirulina group (mean difference=6.30; p<0.001), suggesting an enhancement of erythropoietic activity. Mean corpuscular volume (MCV) was significantly increased following Spirulina supplementation (p<0.001). Mean corpuscular hemoglobin (MCH) was statistically significant difference between the two groups (p<0.001). However, mean corpuscular hemoglobin concentration (MCHC) was significantly higher in the Spirulina-supplemented rats compared to controls (mean difference=0.99; p=0.020). Platelet (PLT) counts were statistically significant between the groups (p <0.001).

Figures 1-3 visually illustrate the effects of Spirulina supplementation on selected hematological parameters, highlighting the significant differences observed in red blood cell indices between the two groups.

Table-I: Effects of two different concentrations of *Spirulina* on blood elements

Blood elements	Control Mean ± SD (n=21)	Spirulina platensis, 400mg Mean ± SD (n=21)	Mean difference	p-value
WBC	21.28 ±4.30	24.59 ± 2.95	3.37	0.007
RBC	6.79 ± 0.87	8.93 ± 0.89	2.14	<0.001
Hb	14.39 ± 1.64	20.69 ± 2.79	6.30	<0.001
MCV	56.28 ± 2.49	59.17 ± 2.18	2.89	<0.001
MCH	21.12 ± 1.19	23.13 ± 1.41	2.01	<0.001
MCHC	37.49 ± 1.23	39.48 ± 1.41	1.99	<0.001
PLT	695.32 ± 137.64	787.71 ± 143.67	92.39	0.040

*p-value was obtained from 't'-test

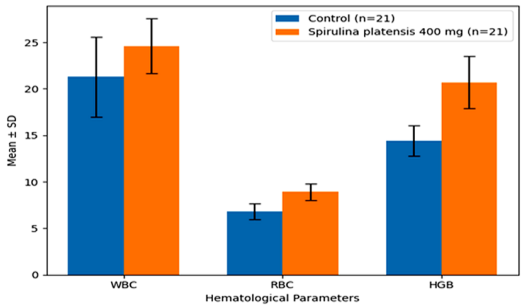

Figure-1: Comparison of hematological parameters between Control and Spirulina platensis (400 mg) groups

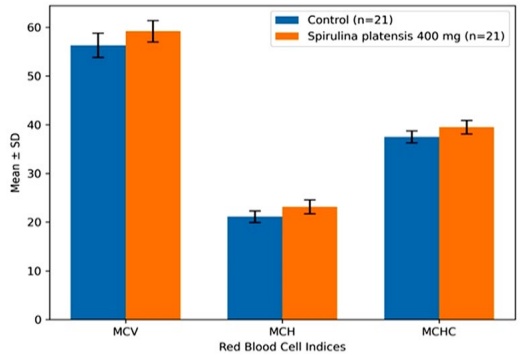

Figure-2: Comparison of MCV, MCH, and MCHC between Control and Spirulina platensis (400 mg) groups

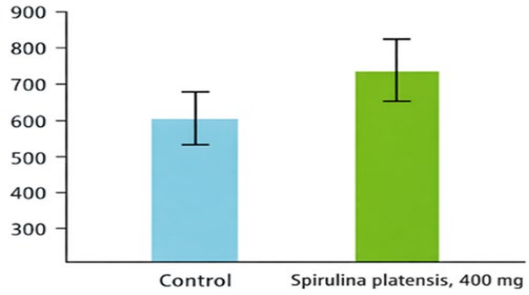

Figure-3: Platelet concentrations between Control and Spirulina platensis (400 mg) groups

Discussion

The present study evaluated the effects of oral *Spirulina platensis* supplementation on hematological indices and selected biochemical parameters in Long-Evans rats. The findings demonstrated that administration of Spirulina at a dose of 400 mg/kg body weight significantly increased red blood cell (RBC) count, hemoglobin (Hb) concentration, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), white blood cell (WBC) count or platelet (PLT) count compared with the control group. These findings were consistent with previous studies[1,5-8]. These results suggest that Spirulina supplementation predominantly enhances erythropoietic indices

without exerting notable effects on leukocyte or platelet parameters.

The observed improvements in RBC-related parameters may be attributed to the rich nutritional profile of Spirulina, which includes high-quality proteins, iron, vitamins, essential amino acids, and antioxidant compounds. These nutrients play critical roles in erythropoiesis and hemoglobin synthesis. Hematological indices are widely recognized as sensitive markers of physiological and nutritional status, and the significant elevations in RBC, Hb, MCH, and MCHC observed after 28 days of *Spirulina* intake indicate a favorable effect on red blood cell production and hemoglobin content.

The results of the present study are in agreement with findings reported in several earlier studies. Ibraheem, et al.[20] demonstrated significant increases in red blood cell count and hemoglobin-related parameters following *Spirulina* supplementation in male albino mice. Similarly, Nasirian, et al.[21] reported improvements in RBC, WBC, MCHC, PCV, and MCV values in diabetic rats treated with *Spirulina*. Moreover, Gaafar, et al.[22] observed dose-dependent increases in multiple hematological parameters in cattle supplemented with *Spirulina*.

Conversely, some studies have reported increases in WBC counts following *Spirulina* supplementation, particularly at higher doses or with longer treatment durations. Abdulrahman, et al.[23] observed elevated WBC counts in fish fed Spirulina-enriched diets, a finding that contrasts with the present study. Such discrepancies may be explained by differences in species, dosage, duration of exposure, experimental design, and baseline physiological conditions.

Conclusion

In conclusion, the findings of the present study indicate that oral supplementation with *Spirulina platensis* at a dose of 400 mg/kg body weight exerts beneficial effects on red blood cell indices in Long-Evans rats. These results support the potential role of *Spirulina* as a functional dietary supplement for enhancing hematological status.

Recommendations

Further studies with larger sample sizes and varying doses of *Spirulina platensis* are recommended to establish dose-response relationships and long-term safety. Additionally, molecular and mechanistic studies, as well as clinical trials, are warranted to validate its hematological benefits and potential therapeutic applications.

Conflict of Interest

The authors declared that they have no conflicts of interest.

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