

Efficacy of Dried Green Algae on Hematological Parameters and Body Weight of Obese Anemic Rats

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Abstract

Obesity is a risk factor for several comorbidities and complications, this includes iron deficiency anemia. The purpose of this study was to investigate the effects of dried green algae (*Spirulina platensis*) on hematological parameters of obese anemic rats. The current study was performed on 35 adult male rats. Rats (n=35) were divided into two main groups. The first group (7rats) was fed on basal diet as negative control group. The second group (28 rats) were fed on high fat diet, deficient in iron and adding 10 g tannic acid/kg diet to cause obese anemic model then were divided into 4 subgroups, the 1st subgroup was fed on high fat diet and kept as a control positive group while, other subgroups 3, 4 and 5 were fed on high fat diet and supplemented with 2.5%, 5%, and 7%, dried spirulina, respectively. Supplementation with spirulina dried at the tested levels overcome (P<0.05) obesity and cause improvement in hematocrit, hemoglobin, red blood cells and platelets of the treated groups. In conclusion, data suggested that spirulina may be useful for obese anemic patients, so it is worthy trial on humans.

Key words: *Spirulina platensis*, body weight gain, hematological parameters.

Introduction

Obesity is defined as an abnormal or excessive fat accumulation that presents a risk to health. The main cause of weight gain and obesity is the imbalance between the amount of Calories intake and the burned Calories. (*Chooiet al., 2019*). According to the recent report by the WorldHealth Organization (WHO), in 2014 more than 600 million adult were obese. Overweight and obesity are the fifth leading risks for global deaths (*WHO, 2014*).

The classification of obesity relies on adult BMI standards. It is a measure of weight adjusted for height, calculated as weight in kilograms divided by the square of height in meters (kg/m²). BMI is an important indicator as a factor related to the risk of maternal diseases such as diabetes mellitus, cardiovascular disease and hypertension. (*Zhaoetal.,2015*).

Anemia is a condition in which you lack healthy red blood cells that transfer proper amount of oxygen to the all parts of body (*Contaldo et al., 2019*). Anemia is characterized by reduced concentrations of hemoglobin (Hb) in the blood below normal levels, /or a diminished number of red blood cells (RBC) (*Cappelliniand Motta, 2015*).

Dietary deficiencies play role in the development of many types of anemia, including iron, cobalt, magnesium, and micronutrients, including vitamin A, folate, B6, B12, and other B vitamins are directly required for the formation of RBC, Hb synthesis, and iron absorption, as well as antioxidant defense and cellular energy (*Abbaspour et al., 2014*).

Iron deficiency anemia (IDA) underlies the majority of anemia cases, affecting 1.6 billion people, which comprise 24.8% of the world population. Iron deficiency anaemia (IDA) is the major anemia condition around the globe that affects more than 6 million individuals of all segments of the population and contributes to approximately 840,000 deaths (*Da Cunha et al., 2019*).

Spirulina, or correctly *Arthrospira* spp., is one of the most important microalgal groups currently produced and contains macro- and micronutrients such as high-quality proteins, minerals, vitamins, fatty acids, polysaccharides and other bioactive compounds (*Al-Dhabi and Arasu, 2016*). Spirulina (SP) is a blue-green algae belonging to the *Cyanobacteria* family that is rich in bioactive compounds such as pigments (phycocyanin, β -carotene) riboflavin, tocopherol and α -linoleic acid (*Costa et al., 2019*). There are two species of Spirulina including *Spirulina platensis* and *Spirulina maxima*. (*Tefera et al., 2016*). Spirulina species have anti oxidative effects, and radical scavenging properties the nutritional benefits of Spirulina should be adequately recognized and utilized (*Abdel-Daim et al., 2013*).

Materials and Methods

Materials:

Chemicals: Casein, vitamins, minerals and cellulose were purchased from El-Gomhoria Company, Cairo, Egypt. Tannic acid was purchased from local distributor of (Sigma Chemical Co) Cairo, Egypt. Kits for blood analysis were purchased from Alkan Company for Bio diagnostic Reagents, Dokki, Cairo, Egypt.

Spirulina: *Spirulina platensis* samples were obtained from the Biotechnology Unit, National Research Centre, Dokki-Cairo, Egypt. Beef tallow was obtained from local market.

Rats: Adult male albino rats (Sprague- Dawley strain) (n=35 rat) weighing approximately (170 \pm 5 g.) were purchased from Helwan Experimental Animals Farm.

Methods:

Identification of *Spirulina platensis* was carried out at the Agriculture Research Center, Kingdom: Bacteria, **Subkingdom:** Gracilicutes, **Phylum:** Cyanobacteria, **Class:** Cyanophyceae, **Subclass:** *Oscillatoriothycideae*, **Order:** Spirulina, **Family:** Spirulina, **Genus:** *Spirulina* spp.

Chemical composition: The gross chemical composition and phytochemical screening were carried according to the official methods (*Alagbe et al., 2020*).

Induction of obesity and anemia: Rats were fed on basal diet according to (*Reeves, et al., 1993*) with high in fat diet (HFD) and low in iron to induce obese and anemic rats (*Liu, et al., 2004*).

Preparation of Spirulina powder: After collection, *Spirulina platensis* samples were washed with fresh water several times to remove salts and debris, and then was air dried at Solar Energy Unit, at the National Research Centre.

Biological study: Thirty-five adult rats were fed on basal diet for one week for adaptation. Rats then were randomly divided into two main groups as follow: The first main group (n= 7) was fed on basal

diet only and served as control negative group. The second main group (n=28) was fed on high-fat diet with 10% tannic acid and iron-was excluded from the mineral mixture to cause obese anemic rats, then were divided into four subgroups, the first subgroup was fed on a high-fat basal diet with tannic acid and served as positive control group, the other three subgroups were fed on high-fat basal diet with tannic acid supplemented with dried Spirulina at the level of 2.5, 5 and 7 %.

At the end of the experimental period (6 weeks), rats were fasted over night before sacrificing, two blood samples were collected, and the first sample was collected into a tube containing disodium salt of Ethylene Diamine Tetra Acetic Acid (EDTA) as anticoagulant and used for assessment of the hematological parameters. The second blood samples were collected into a centrifuge tube without any anticoagulant and centrifuged to obtain serum which was stored at - 20°C until used for subsequent analysis.

Calculation of body weight Gain (BWG %) and feed efficiency ratio (FER).

Daily feed intake was calculated day after day throughout the experimental period (6 weeks). Calculation of BWG % and FER, were assessed according to the method described by **Chapman et al., (1959)** using the following equations:

$$\text{BWG}\% = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100$$
$$\text{FER} = \frac{\text{weight Gain (g)}}{\text{Feed intake (g)}}$$

Chemical analysis:

Total red blood cell counts (RBCs), hemoglobin (Hb), hematocrit (HCT) and platelets were estimated according to **(Dacie and Lewis, 1998)**. Leptin hormone was determined using enzyme-linked immune sorbent (ELISA) assay **(Considine et al., 1996)**.

Statistical Analysis: The obtained results were analyzed according to SPSS program. ANOVA test was used to compare results among groups and (P<0.05) was the significant difference **(Senedecor and Cochran, 1989)**.

Results and Discussion

Chemical composition of spirulina platensis Table (1) :

Spirulina platensis contains crude protein, total lipids, total carbohydrate, crude fiber, ash and total antioxidant activity, by 56.79g, 8.33g, 13.60g, 4.25g, 10.05g, and 39.2µg/100 g of the dried leaves, respectively. Flavonoids(11.1 µg) and phenolic acid (997.1 µg). Iron was the most micronutrients found but we also found zinc and calcium. The percentage of phycocyanin(16.15%) while carotenoids were (3.8%) **(El-Moataaz et al., 2019)**. Similar to this study **(Sharoba, 2014)** found that Spirulina is a rich source of protein as it contains 62.84% of its weight protein mostly 38.46% essential amino acids. Besides, Spirulina is a perfect source of beta-carotene and vitamin E **(Gutiérrez-Salmeán et al., 2015)**.

Table (1):

Chemical composition and total antioxidant activity of spirulina platensis

Nutrient (/100g)	Amount
Protein	56.79 g
Carbohydrates	13.60 g
Fibers	4.25 g
Calcium	363.7 mg
Iron	12.4 mg
Zinc	2.6 mg
B-Carotene	70.0µg
Vitamin E	60.0 mg
Phenolic compounds	997.1 µg
Flavonoids	11.1 µg
Total antioxidant activity	39.2µg

Effect of spirulina platensis onBody Weight Gain(BWG %) and feed efficiency ratio (FER) of obese anemic rats (table 2).

There was a significant increase in the initial body weight of all groups of rats as compared to the -ve control group. Obese anemic rats treated with different levels of spirulina platensis had significant ($P<0.05$) decrease in final body weight (FBW), body weight gain (BWG %) and feed efficiency ratio(FER)as compared to the positive group (obese anemic rats).The mean values of the feed intake (FI) of the control (-ve) and control (+ve) groups were (17 and 20 g/d), respectively. When rats were fed on Spirulina with different tested levels, their feed intake (FI) decreased, as compared to the positive control group.There were significant differences among the three tested groups in BWG% and FER. These results agreed with **El-Hashash, (2021)**, who revealed that all hypercholesterolemic rats which fed on Spirulina had significant decrease in body weight gain.

Likewise, oral administration of S. platensis aqueous extract to diabetic rats for 50 days led to an obvious regain in their body weight loss, suggesting general health status and metabolic mechanisms improvement (**Aissaouiet al., 2017 andHussainiet al., 2018**).however, **Ismail et al., (2020)** showed that Spirulina platensis improved the body weight losses compared to diabetic rats after six weeks treatment.Also,**Khateib, (2021)** showed that treatment with spirulina can increase FI, FER and BWG of anemic rats.

Table (2):

Effect of spirulina platensis leaves powder on Body Weight Gain (BWG %) and feed efficiency ratio (FER) of obese anemic rats

Parameters Groups	IBW (g)	FBW(g)	BWG g%	FI (g/d/rat)	FER
Control (-ve)	165.0±1.15 ^b	191.3±2.60 ^b	15.95±1.31 ^b	17.0	0.034±0.02 ^b
Control (+ve)	200.0±1.73 ^a	248.0±1.15 ^a	24.02±1.45 ^a	20.0	0.053±0.02 ^a
Spirulina 2.5%	197.3±0.88 ^a	169.6±0.88 ^c	-14.01±0.78 ^c	15.0	-0.041±0.02 ^c
Spirulina 5%	200.6±1.20 ^a	157.7±2.90 ^d	-21.44±0.98 ^d	12.3	-0.078±0.03 ^d
Spirulina 7%	201.7±1.45 ^a	133.0±1.52 ^e	-34.03±1.01 ^e	11.0	-0.139±0.04 ^e

Results are expressed as mean ± SE. Values in each column which have different letters are significantly different at (P<0.05).

Effect of spirulina supplementation on Hemoglobin (Hb) and Hematocrit (HCT) levels of obese anemic rats(table 3).

The obtained results showed that rats fed on high fat diet deficient in iron with added tannic acid revealed a significant decrease (p<0.05) in the mean value of RBC, Hb and HCT compared with those of the negative control group. Obese anemic rats fed on spirulina at the different levels had significant (P<0.05) increase in RBC, Hb and HCT compared with that of the positive control group. Spirulina at (7%) had highest significant increment in RBC, Hb and HCT than the other groups.

The obtained results agreed with the results of *Purikhet et al., (2013) and Ramesh et al., (2013)* who observed a significant increase in hemoglobin when used spirulina powder for five weeks. Also, *Bléyééré et al., (2013)* showed that consumption with spirulina by rabbits led to an increase in the levels of red blood cells and hemoglobin and there was a steady increase in the level of hemoglobin. *Kambouet et al., (2015)* studied anti anemic effect of spirulina in rabbits and showed that spirulina is a rich source of nutrients. *Roberto, (2015)* and *Balasubramani et al., (2016)* showed that spirulina contains minerals such as iron, magnesium, calcium, and phosphorus. Spirulina is a splendid source of iron which contains 20 times more iron than wheat so spirulina may help cases of anemia. *Abed et al., (2016)* showed that spirulina improved hemoglobin level in children blood when used for 12 weeks.

These results are supported by the results published by *Visnegarwala and Mahesh, (2017)* showed the effects of spirulina, blue green algae, as an alternative to iron supplements, not only alleviate the anemia of pregnancy but also have impact on the fetal and maternal outcomes, through its impact on the gut micro biome. Being good source of iron Spirulina was useful for anemic persons and excellent for women during pregnancy (*Radha and Chandra, 2018*). *Khateib, (2021)* showed that treatment with spirulina can increase RBC, Hb and Hematocrit of anemic rats.

Table (3):

Effect of spirulina on Hemoglobin and Hematocrit levels of obese anemic rats.

Parameters Groups	RBC (10 ⁶ /ml)	Hb (g/dl)	Hct (%)
Control (-ve)	9.73±0.09 ^a	14.45±0.19 ^a	49.07±1.90 ^a
Control (+ve)	4.89±0.16 ^d	7.88±0.17 ^e	25.36±0.96 ^e
Spirulina 2.5%	6.41±0.18 ^c	8.86±0.08 ^d	31.95±1.15 ^d
Spirulina 5%	7.69±0.12 ^b	10.20±0.14 ^c	38.24±0.64 ^c
Spirulina 7%	9.17±0.40 ^a	11.29±0.19 ^b	44.43±1.02 ^b

Results are expressed as mean ± SE. Values in each column which have different letters are significantly different at (P<0.05).

Effect of spirulina supplementation on Leptin hormone of obese anemic rats (table 4):

Rats fed on high fat diet revealed a significant increase (P<0.05) in the mean of leptin hormone, as compared with those of negative control group as seen in table (4). Rats fed on high fat diet (HFD) supplemented with spirulina at the three tested levels had significant decrease (P<0.05) in the level of serum leptin, as compared with the positive control group. There was significant difference in the level of leptin among the three groups treated with spirulina. The highest reduction for leptin hormone was recorded at the group supplemented with 7% of spirulina.

The present results agree with **Makoto et al., (2012)** who revealed that Spirulina was associated with significant decrease in leptin, as compared to the control group. Also, with **Myeong and Se, (2018)** who found that the value of leptin significantly increased in high-fat diet. However Spirulina maxima (SM), supplementation decreased the level of leptin hormone. **El-Soadaa and Negm (2019)** showed that, rats fed high fat diet revealed a significant increase (P<0.05) in the mean of leptin hormone, compared with those of negative control group

Table (4):

Effect of spirulina supplementation on Leptin hormone of obese anemic rats.

Parameters Groups	Leptin (µ/L)
Control (-ve)	9.59±0.35 ^e
Control (+ve)	30.97±0.83 ^a
Spirulina 2.5%	21.16±0.89 ^b
Spirulina 5%	17.60±0.67 ^c
Spirulina 7%	13.88±0.33 ^d

Results are expressed as mean ± SE. Values in the column which have different letters are significantly different at (P<0.05).

Conclusions

the results denote that different levels of Spirulina improved decreased body weight, and improved hemoglobin level. So, the present study recommends trial intake of Spirulina supplement for patients who suffer from obesity and anemia.

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فعالية الطحالب الخضراء المجففة في معايير الدم ووزن الجسم للفئران المصابة بالسمنة وفقر الدم

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الملخص العربي

السمنة هي عامل خطر للعديد من الامراض والمضاعفات، ومن هذه المضاعفات الإصابة بالانيميا الناجمة عن نقص الحديد. كان الغرض من هذه الدراسة هو دراسة تأثير الطحالب الخضراء المجففة (سبيرولينا بلاتنسيس) علي مقاييس الدم وتأثيرها علي وزن الجسم لفئران المصابة بفقر الدم والسمنة أجريت الدراسة على 35 فئران بالغة وزنها 170 ± 5 جم. تم توزيع المجموعات عشوائيا إلى خمس مجموعات كل منها عدد 7 فئران . كانت المجموعة الأولى ضابطة سالبة ، بينما المجموعة الثانية تحتوي علي (28 فار) تم تغذيتها على نظام غذائي عالي الدهون ، منخفض في الحديد ومكمل بـ (10 جم حمض التانيك / كجم) ثم قسمت إلى 4 مجموعات فرعية ظلت المجموعة الثانية مجموعة ضابطة موجبة، بينما تم إعطاء المجموعات الثالثة والرابعة والخامسة على النظام الغذائي الأساسي المضاف إليه 2.5% و 5% و 7% سبيرولينا على التوالي. في نهاية التجربة تم جمع عينات الدم. تم حساب الماخوذ من الطعام ونسبة كفاءة التغذية وزيادة وزن الجسم. الهيموجلوبين في الدم وخلايا الدم الحمراء الهيماتوكريت. اشارت النتائج ان الطحالب الخضراء المجففة تساعد في علاج الانيميا الناتجة عن نقص الحديد , وهذا يرجع الي تحسن في متوسط الهيماتوكريت والهيموجلوبين وخلايا الدم الحمراء والصفائح الدموية للمجموعات المعالجة مقارنة بالمجموعة الضابطة الموجبة. علاوة علي ذلك , لوحظ انخفاض معنوي في الوزن لذلك , توصي الدراسة بتجربة ذلك علي مرضي السمنة و يعانون بالانيميا.

الكلمات المفتاحية: سبيرولينا بلاتنسيس ، زيادة وزن الجسم ، بارامترات أمراض الدم .