The Role of Spirulina platensis (*Dried Green Algae*) in Weight Reduction of Obese Anemic Rats

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Abstract

Obesity has been related with disordered iron homeostasis and cause anemia.The present study was carried out to investigate the effect of spirulina platensis (Dried green algae) on hematological parameters and its role on body weight 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 subgroupwas fed on high fat diet and kept as a control positivegroup 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 dried spirulinaat the tested levels might help overcome ($P<0.05$)obesity due to the improvement in hematocrit, hemoglobin, red blood cells and platelets of the treated groups. Moreover, significant ($P<0.05$) improvement in liver functions and lipid profile of the tested rats were observed. In conclusion, data suggested that spirulina is useful for obese anemic patients.

KeyWords: Spirulina platensis, body weight gain, blood cholesterol, hematological parameters.

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Introduction:

Obesity prevalence will reach 50% by 2030 **(Ward et al., 2019).** The current prevalence of obesity is 42% and the severe adult obesity (BMI > 40) has increased recently **(Hales et al., 2020).** Obesity is related to many chronic complications, as hormonal imbalance, hyperglycemia, dyslipidemia, impaired glucose tolerance **(Redha et al., 2021).**

Anemia is a medical status associated with decreased or increased red blood cells characterized by insufficient oxygen-carrying capacity to fill physiological requirements **(Marks, 2019).** The most famous reason of anemia is deficiency of iron; iron being an integral portion of the blood protein, hemoglobin. However, there are other abnormalities also associated with anemia like shortage of vitamin A, vitamin B12, chronic inflammation and parasitic infections **(Premkumar et al., 2018).**

Low iron availability results in the medical condition, hypochromic microcytic anemia due to low hem concentration in the blood **(Moradi, et al., 2019).**Nowadays, therapeutic drugs caused many side effects; therefore, using foods from natural plants could exist a suitable solution for treating anemia **(Onyeabo et al., 2017).**

Medicinal plants have long been used against life threatening diseases including diabetes. Some of these plants have been shown to possess antioxidant activities, which could help improving diabetes inconveniences **(Gargouri et al., 2016).** Spirulina (SP) is a blue-green algae belonging to the Cyanobacteria family that is rich in bioactive compounds such as proteins, lipids, carbohydrates, trace elements, 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. Spirulina is the world's largest natural protein source and an important medicinal herb **(Tefera et al., 2016).**Spirulina species have ant 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 distributer of (Sigma Chemical Co) Cairo, Egypt. Kits for blood analysis were purchased from Alkan Company for Biodiagnostic 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 ±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: Oscillatoriophycideae, 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 model: Rats were fed four weeks on basal diet according to (Reeves, et al., 1993) with some modification in fat content (HFD) for four weeks and the remainder was starch to induce obesity in rats **(Liu, et al., 2004).**

Induction of anemia: Obese rats were continuously fed on high fat diet supplemented with (10 mg tannic acid/kg diet) for 3 weeks **(Afsana et al., 2004)** at the same time, iron was removed from the mineral mixture to induce anemia. Blood samples were obtained after 3 weeks from tail vein to determine Hemoglobin level**(Borzage et al., 2016).**

Preparation of Spirulina powdered: 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-removed 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:

BWG%=(Final body weight – Initial body weight)/(Initial body weight)× 100

FER=(weight Gain (g))/(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).** Serum Aspartate amino transferees (AST) and alanine amino transferees (ALT) **(Retimanand Frankel, 1957)** were determined. Serum total cholesterol (TC)**(Trinder, 1969),** triglycerides **(Wahlefeld, 1974),** high density lipoprotein **(Albers et al., 1979)** were determined. Meanwhile, low density lipoprotein and very low density lipoprotein were calculated according to **(Friedwald et al., 1972).**

LDL-c = TC-[HDL-c + (TG/5)], VLDL-c = TG/5

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 significant **(Senedecorandcochron, 1989).**

Results and Discussion

Table (1) chemical composition of spirulina platensis: 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 39.2 µg/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).** The findings of the instant study agreed with the results of **(Ragheb and Aljehany, 2020)** who reported that Spirulina contains high protein percent (57.30 %) of its dry weight. Moreover, it contains high amount of vitamin E and β-carotene. Similar to this study **(Sharoba, 2014)** who 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
Ash	10.05 g
Lipids	8.33 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

Table (2) Effect of spirulina platensis leaves powder on BWG, FI, and FER of obese anemic rats.

The effect of spirulina platensis on body weight of obese anemic rats are recorded in **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 FBW, BWG% and FER as compared to the positive group (obese anemic rats).The mean values of the (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 FI were decreased, as compared to the positive control group. There were significant differences among the three tested groups in BWG% and FER. These results are agreed with **El-Hashash, (2021),**who revealed that all hypercholesterolemia 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 **(Aissaoui et al., 2017 and Hussaini et 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 BWG, FI, and FER of obese anemic rats.

Parameters / Groups	IBW (g)	FBW(g)	BWG%	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).

Table (3): Effect of spirulina supplementation on Hemoglobin and Hematocrit levels of obese anemic rats.

The obtained results showed that rats fed on high fat diet deficient in iron and added with tannic acid revealed a significant decrease (p<0.05) in the mean value of RBC, Hb and HCT as compared with those of the negative control group as shown in **Table(3).**Obese anemic rats fed on spirulina at the different levels had significant (P<0.05) increase in RBC, Hb and HCT as compared with that of the positive control group. It was clear that, there was significant difference in RBC, Hb and HCT among all the experimental groups. Spirulina at (7%) had high significant increment in RBC, Hb and HCT than the other groups.

The obtained results are agreed with the results of **Purikh et al., (2013)** and **Ramesh et al., (2013)**who observed a significant increase in

hemoglobin when used spirulina powder for just five weeks, so spirulina could be used in treatment of iron deficiency anemia. Moreover,**Pugazhendy et al., (2012)** described a significant increase in hemoglobin levels in patients who are anemic. Also, **Bléyéré et al., (2013)**showed that consumption with spirulina led to an increase in the levels of MCH, red blood cells and hemoglobin and there was a steady increase in the level of hemoglobin. **Kambou 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 gram so spirulina is a good treatment for anemia.**Abed et al.,(2016)**showed that spirulina improve hemoglobin level in the 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,to not only alleviate the anemia of pregnancy but also have impaction the fetal and maternal outcomes, through its impact on the gut micro biome. Spirulina is useful for anemic persons because it is a good source of iron, meaning it is 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. **Negm et al., (2021)**showed that rats fed basal diet deficient of iron and added with tannic acid revealed a significant decrease ($p<0.05$) in the mean value of Hb and HCT as compared with those of negative control group.

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 ($P<0.05$).

Table (4) showed the effect of spirulina on liver functions of obese anemic rats. Rats fed high fat diet (positive control group) revealed a significant increase ($p<0.05$) of liver functions as compared with those of the negative control group. Supplementation with spirulina at the tested levels caused a significant decreased ($P<0.05$) in serum AST, ALT and ALP as compared to the positive control group. There was a significant difference in AST and ALT among all the treated groups with spirulina. The highest improvement in AST, ALT and ALP was observed at the group supplemented with spirulina at 7%.The results agreed with **Ragheb and Aljehany, (2020)** who found that consuming Spirulina in ascending doses caused a significant decrease in the levels of liver enzymes as compared to rats in the PTU group. In agreement with the results of the current study, the researchers advised that consuming Spirulina supplement daily for a period of 6 months had reduced elevated liver enzymes associated with non- alcoholic fatty hepatitis. Also, the effect of Spirulina was associated with a decreased concentration of blood fat as well**(Ferreira et al., 2010 and Mazokopakis et al., 2014).**

Moreover, spirulina improved virus C hepatitis and reduced liver enzymes in β- thalassemia major diseased children by decreasing serum ferritin and ameliorated immunity**(Elshanshory et al., 2020).**The hepato-protective mechanism of Spirulina against liver damage induced in rats and humans is due to the richness of this Algae with antioxidants and anti-inflammatory including vitamin E, Cphycocyanin, and β-carotene, as well as its ability to get rid of excess liver lipids**(Park et al., 2008and Deng and Chow, 2010).** Moreover, **Mazloomi et al., (2021)** demonstrated that fatty liver grade decreased significantly in the Spirulina group in comparison with the placebo group.

Table(4): Effect of Spirulina platensis supplementation on liver functions in obese anemic rats.

Parameters / Groups	ALT	AST	ALP
	(µ/L)		
Control (-ve)	35.80±0.65^e	68.79±0.93^e	71.84±1.96^c
Control (+ve)	61.00±2.17^a	102.41±1.23^a	114.93±2.14^a
Spirulina 2.5%	53.20±0.81^b	89.86±2.12^b	93.50±1.32^b
Spirulina 5%	46.17±0.58^c	80.40±0.67^c	88.61±1.92^b
Spirulina 7%	39.37±0.85^d	74.16±0.71^d	74.67±1.93^c

Results are expressed as mean ± SE. Values in each column which have

different letters are significantly different at (P<0.05).

Table (5): Effect of spirulina supplementation on Leptin hormone of obese anemic rats.

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 (5).** Rats fed on 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 a 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,**Myeong and Se, (2018)**found that the value of leptin was 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, as compared with those of negative control group.

Table (5): 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 each column which have different letters are significantly different at (P<0.05).

The obtained results from **Table (6)** showed that the control positive group had a significant increase (P<0.05) in the mean values of serum TC, TG, LDL-c and VLDL-c, while the mean value of HDL-c was significantly decreased as compared with the control negative group. Obese anemic rats fed on spirulina at the three tests levels had significant decrease (P<0.05) in serum TC, TG, LDL-c, VLDL-c, while serum HDL-c was significantly increased. The highest improvement in lipid profile was observed at the group supplemented with 7% spirulina. These results are in a line with **Haohai et al., (2018)**who found a decreased in the levels of TG,TC,LDL-c and VLDL-c and increased HDL level in the Spirulina supplemented groups.

Evidence suggests that consumption of spirulina as a dietary supplement prevents hypercholesterolemia due to the large amount of cysteine found in the Cphycocyanin protein in spirulina **(Cheong et al., 2010).** Some authors suggest that the addition of this alga into the diet diminishes the intestinal absorption of cholesterol as well as the re-absorption of bile acids in the ileum. Thus, they suggest that spirulina can be considered a functional food capable of reducing weight **(Gargouri et al., 2016).** These results confirmed with **Mehdinezhad et al., (2021)** who demonstrated that treatment with Spirulina resulted in a marked improvement in lipid profile. In agreement with the results of the current study, the researchers advised that consuming Spirulina supplement was associated with a decreased concentration of blood fat **(Mazokopakis et al., 2014).Gheda et al., (2021)** showed that S. platensis methanolic extract decrease in hyperlipidemia related to diabetes were also detected. The lipid-lowering mechanism for this Alga lies in its ability to reduce the level of triglycerides as studies have proven to contain a high level of protein and very little fat **(Ragheb and Aljehany, 2020).**

Table(6): Effect of Spirulina platensis supplementation on lipoproteins levels in obese anemic rats.

Parameters / Groups	TC	TG	VLDL-C	HDL-C	LDL-C
	(mg/dl)				
Control (-ve)	76.15±2.45^{d}	48.00±1.47^{e}	9.60±0.29^{e}	56.73±1.89^{a}	9.82±1.99^{d}
Control (+ve)	114.10±2.52^{a}	82.76±1.37^{a}	16.55±0.27^{a}	25.83±1.50^{e}	71.71±3.97^{a}
Spirulina 2.5%	101.93±1.67^{b}	73.49±1.78^{b}	14.69±0.35^{b}	36.10±1.44d	45.33±1.95^{b}
Spirulina 5%	92.06±1.50^{c}	68.50±1.02^{c}	13.70±0.20^{c}	41.90±1.38^{c}	42.26±2.02^{b}
Spirulina 7%	87.00±0.70^{c}	57.16±2.28^{d}	11.43±0.45^{d}	48.58±2.37^{b}	26.98±2.66^{c}

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

Conclusions:

The results denotethose different levels of Spirulina improved decreased body weight, and improved liver function and serum lipid profile as well as improving hemoglobin level. So, the present study recommends that intake of Spirulina supplement may be beneficial for patients who suffer from obesity and help anemia.

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