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Some pharmacological studies on *Chlorella vulgaris* in tilapia fish

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Abstract

Objective: This study was undertaken to evaluate the therapeutic effect of *Chlorella vulgaris* microalgae on Nile tilapia (*Oreochromis niloticus*).
Methods: A total of 40 healthy fish were used in this study and were randomly classified into two groups (n= 20/group). The 1st group served as a control, while the 2nd group received *C. vulgar* powder (5%) in feed for one month. Blood samples were collected post-treatment. The serum samples were used to estimate the levels of total protein, albumin, globulin, lipid profile (total cholesterol, triglycerides, high-density lipoprotein, and low-density lipoprotein), uric acid, creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Also, some hematological parameters were measured post-treatment.
Results: There was a significant increase in serum levels of total protein, albumin, and globulin, while total cholesterol level was significantly decreased in the *C. vulgaris* group as compared to the control group. The levels of AST, ALT, uric acid, and creatinine showed no significant changes. Moreover, the supplementation of *C. vulgaris* in the diet of treated fish evoked a significant increase in hemoglobin, packed cell volume %, red blood cells, and white blood cells count.
Conclusion: These findings conclude that the addition of *C. vulgaris* algae in fish diet enhances the health status of Nile tilapia without any negative effects on blood parameters.

Keywords: Chlorella vulgaris, Nile tilapia, Hematological parameters, Algae.

1. Introduction

Fish is one of the cheapest and excellent sources of high-quality animal protein, fatty acids, and many essential nutrients. They can be used also as a model for experimental studies (Mahfouz et al., 2015). Tilapia is an important commercial freshwater fish species that spreads worldwide and plays an important role in fisheries aquaculture as a source of animal proteins (Grammer et al., 2012; Sharawy et al., 2017).

Feed additives are used in fish farms to maintain the nutritional value of the feed ingredients before feeding. They also improve feed ingestion, increase consumer acceptance of the product, and promote growth (El-Adawy et al., 2018). So, the potential use of natural unconventional feed additives such as algae, for substitution the high-cost foodstuff such as fish meal is very important for the aquaculture industry today (Radhakrishnan et al., 2015). In fish farms, algae are used as a good alternative source of protein, vitamins, and essential fatty acids. The main microalgae used as a feed additive are *Chlorella*, *Chaetoceros*, and *Pavlova*. They contain all nutrients such as essential proteins, lipids, carbohydrates, vitamins, minerals, amino acids, and fatty acids and therefore they are known as living capsules of nutrition (Ebru and Cengiz, 2016). Chlorella is one of the most well-known microalgae that contain many natural bioactive compounds. It is used in food and pharmaceutical industries. Chlorella contains many important components such as Omega-3 and Omega-6 polyunsaturated fatty acids, amino acids, polysaccharides, α- and β-carotene, some minerals, pro-vitamins, vitamins, chlorophyll, and lutein (Radhakrishnan et al., 2014). It can also be used as an immunomodulator, anticancer, and therapeutic adjuvant in the treatment of age-related diseases (Mahmoud et al., 2018).

This work aimed to investigate the effects of *Chlorella vulgaris* on health-related parameters in Nile tilapia (*Oreochromis niloticus).*

2. Materials and methods

2.1. Chlorella vulgaris

C. vulgaris microalgae in the form of dried green powder was purchased from the Institute of National Research Center, Cairo. The diet ingredients were mixed with oil and then with water to obtain a dough. Then, the dough was passed through an electrical mincer for pelleting. The pellets were dried at room temperature for a few days and crushed to yield fine particles.

2.2. Aquaria

Two glass aquaria measuring 50×50×100 cm were used in this study. They were provided by aerating devices, heaters, and fresh dechlorinated tap water. The aquaria were kept in a natural laboratory environment. One-third of the water in each aquarium was daily exchanged with removing excreta and food residues from the bottom of aquaria by siphoning after feeding ceased.

2.3. Fish

A total number of 40 healthy tilapia fish (*Oreochromis niloticus*) were obtained from a private fish farm in Dakahlia Governorate. The weight of fish varied between 55 and 60g. Fish transported alive to the laboratory of Pharmacology Department, Faculty of Veterinary

Medicine, Mansoura University. Fish were acclimatized for 2 weeks before the beginning of the experiments to minimize the possible stress and ensure that fish have been adapted to the laboratory environmental condition.

2.4. Experimental design

Fish were divided into 2 groups (n = 20/group): The control group (G1): fish received diet free from any additives and G2 (*Chlorella vulgaris*): fish received a diet containing 5% *Chlorella vulgaris* powder according to Khani et al., (2016) for one month.

2.5. Blood sampling

At the end of the experiment (30 days), blood samples were collected from the caudal vein in tubes coated with EDTA (whole blood) and plain tubes for serum separation. The obtained sera were stored in a deep freezer at -20^{0}C till assayed.

2.6. Hematological parameters

Erythrocytes and leukocyte count were estimated manually using Natt and Herrick solution. Hemoglobin was determined by the cyanmethemoglobin method using spectrophotometry (540 nm). Packed cell volume (PCV) was measured using the microhematocrit method (Weiss and Wardrop, 2010).

2.7. Serum biochemical analysis

Frozen serum samples were used for estimation of serum aspartate aminotransferase (ALT) and alanine aminotransferase (AST) (Schumann, 2003), total protein, albumin, uric acid, total cholesterol, triglycerides, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) (Young 2001), serum creatinine and globulin (Walker,1990).

2.8. Statistical analysis

The data were statistically analyzed by SPSS (version20, USA). The estimated mean and standards error of mean (SEM) in different groups were carried out using t test as described by Snedecor and Cochoran (1980). The difference between means was considered significant when $P \leq 0.05$.

3. Results

3.1. Effect of Chlorella vulgaris on protein profile

There was a significant increase in serum level of protein, albumin, and globulins in the *C. vulgaris* group compared with the control group (Table1).

Table 1: Effect of *Chlorella vulgaris* on total protein, albumin, and globulin in fish serum.

Groups	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
Control	3.01 ± 0.1^b	1.67 ± 0.05^b	1.33 ± 0.02^b
C. vulgaris	4.78 ± 0.19^a	2.45 ± 0.03^a	2.34 ± 0.19^a

Results were expressed as mean ± standard error of mean (n = 5). In each column, means carried different letters showed a significant change.

3.2. Effect of Chlorella vulgaris on hematological parameters

Supplementation of *C. vulgaris* in diets resulted in a significant increase in Hb, PCV%, RBCs, and WBCs (Table 2).

Table 2. Effect of *Chlorella vulgaris* on hematological parameters in fish.

Groups	RBCs (10^{12}/L)	WBCs (10^9/L)	Hb (g/dl)	PCV (%)
Control	3.24 ± 0.17^b	6.25 ± 0.31^b	9.15 ± 0.13^b	28.41 ± 0.52^b
C. vulgaris	4.78 ± 0.19^a	8.04 ± 0.23^a	13.70 ± 0.15^a	37.46 ± 0.33^a

Results were expressed as mean ± standard error of mean (n = 5). In each column, means carried different letters showed a significant change.

3.3. Effect of Chlorella vulgaris on kidney and liver functions

The obtained data revealed that there was no significant difference in the levels of uric acid, creatinine, ALT and AST in serum of *C. vulgaris*-treated fish in comparison with the control group (Table 3).

Table 3. Effect of *Chlorella Vulgaris* on kidney and liver functions (uric acid, creatinine, ALT and AST) in fish serum

Groups	ALT (U/l)	AST (U/l)	Creatinine (mg/dl)	Uric acid (mg/dl)
Control	18.15 ± 0.18	59.13 ± 0.25	0.47 ± 0.02	1.13 ± 0.01
C. vulgaris	17.88 ± 0.19	57.91 ± 0.98	0.42 ± 0.03	1.08 ± 0.03

Results were expressed as mean ± standard error of mean (n = 5).

3.4. Effect of Chlorella vulgaris on lipid profile

There was a significant decrease in the level of total cholesterol (TC), LDL, and triglyceride in the *C. vulgaris* group while the level of HDL in this group was significantly increased relative to the control group (Table 4).

Table 4. Effect of *Chlorella vulgaris* on lipid profile in fish serum.

Groups	TC (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Triglyceride (mg/dl)
Control	118.73 ± 0.51^a	79.38 ± 0.67^b	12.98 ± 0.61^a	131.82 ± 1.06^a
C. vulgaris	112.68 ± 0.49^b	83.16 ± 0.51^a	4.39 ± 0.80^b	125.59 ± 0.86^b

Results were expressed as mean ± standard error of mean (n = 5). In each column, means carried different letters showed a significant change.

4. Discussion

In the present study, the results revealed a significant increase in total protein, albumin, and globulin in fish fed diet supplemented with *C. vulgaris* powder. These results are similar to those recorded by Khani et al., (2017) and Xu et al., (2014) who found that the addition of *C. vulgaris* for 8 weeks in diets could significantly elevate the total protein, albumin, and globulins in the serum of treated carp. This elevation of serum protein level could be due to the liver function improvement by *C. vulgaris*. Besides, Fadl et al., (2017) found that total protein and globulin were markedly elevated in the serum of Tilapia, after feeding 15% *C. vulgaris* in the diet for 9 weeks. Also, Simanjuntak et al., (2018) found that microalgae supplementation increased total protein, albumin, and globulin compared to controls Gurami fish.

Hematological evaluations are gradually becoming a routine practice for determining health status in fish (Tavares-Dias and Moraes 2006). Our results showed that there is a significant increase in Hb, PCV%, RBCs, and WBCs in 5% *C. vulgaris*-treated fish. An increase in RBCs count can be considered as positive effect made by *C. vulgaris*. Among the immune cell parameters, RBC count is a frequently used parameter to evaluate possible undesired collateral effects such as anemia (Morera et al. 2011). WBCs are the immunocompetent cells of the immune system which play critical roles in both infectious and non-infectious diseases (Magandottir. 2006). This result is agreed with Abdel –Tawab et al., (2008) who stated that a 10 % Spirulina diet increased RBCs and WBCs counts. Furthermore, Sherif., (2012) found that feeding Spirulina microalgae to Nile tilapia at concentrations of 10 and 15 g/ kg diet significantly increased RBCs and higher WBCs count and all groups fed on Spirulina had higher Hb. Also, Khani et al., (2016) examined the effect of *C. vulgaris* on the hematological values of koi Carp for 8 weeks and found that higher values of these blood parameters in treated fish. Moreover, Raji et al., (2018) found that the value of PCV, RBC, and WBC significantly increased after supplementation of the African catfish diet with *C. vulgaris* and *S. platensis* microalgae for 12 weeks. This result is similar to the result reported by Abid., (2018) who revealed that the supplementation of *C. vulgaris* in koi carp feed resulted in a significant increase in RBC, Hb, Hct, and WBC level. Addition of 5 and 7.5 g chlorella/kg diets significantly increase WBC, RBC, and Hb while PCV was highly increased only after supplementation of 7.5g chlorella/kg diet. Also, Simanjuntak et al., (2018) found that supplementation of microalgae in diets for 56 days significantly increased the hematological parameters of Gurami fish. Moreover, Galal et al., (2018) noticed that incorporating *C. vulgaris* in the diet of Tilapia for 15-30 days significantly increased RBC count, Hb concentration, PCV%, and total leukocytic count.

Our results showed that there is no difference in the levels of ALT, AST, creatinine, and uric acid in the serum of treated and untreated fish. Thus, dietary supplementation of *C. vulgaris* did not show any adverse effects on normal physiological conditions. In consistence, Bai et al., (2001) also found no health adverse effect was noticed following feeding Korean rockfish on diet supplemented with *C. vulgaris* for 12 weeks. Moreover, Kim et al., (2002) concluded that the dietary supplementation of *C. vulgaris* powder in the Japanese Flounder commercial diets for 12 wk could make no difference in serum ALT and AST. Khani et al., (2017) also showed that that, ALT and AST levels in serum of treated carp fish had the normal level pattern by dietary addition of *C. vulgaris* for 2 months. Also, Fadl et al., (2017) found that the contribution of 15% *C. vulgaris* enriched fodder for the Nile tilapia did not produce any changes in serum ALT, AST, creatinine, and uric acid. However, Xu et al., (2014) reported an increase in serum levels of ALT and AST in Gibel carp that fed on *C. vulgaris* in the diet for 2 months.

Our study revealed a marked decrease in total cholesterol level, triglyceride, and LDL levels, and a decrease in HDL level in *C. vulgaris*-treated group. This infers that *C. vulgaris* had a lipid lowering effect. Similarly, Kim et al., (2002) found that feeding *C. vulgaris* powder with 2%- 4% in the diet for 12 weeks decreased serum cholesterol levels in juvenile Japanese flounder. Xu et al., (2014) reported that the addition of *C. vulgaris* in the diet for 2 months could minimize cholesterol levels in carp. Moreover, Khani et al., (2017) found that feeding of carp by 5% *C. vulgaris* powder for 2 months resulted in a decrease in serum cholesterol and triglyceride levels. The obtained results agreed with Abid., (2018) who found that the supplementation of common carp with 5% of *C. vulgaris* in the diet resulted in a decrease in the serum levels of cholesterol, triglyceride, and LDL. Raji et al., (2018) also found that increased HDL and decreased LDL levels upon supplementation of African catfish diet with *C. vulgaris* and *S. platensis* for 12 weeks. In support, Abdulrahman et al., (2019) also reported that the addition of *Spirulina* microalgae to common carp diet for 42 days resulted in higher HDL and lower triglyceride and LDL levels.

5. Conclusion

C. vulgaris powder is highly valuable in fish farms as feed additives. It can enhance the health status of Nile tilapia without any adverse effects on normal physiological conditions

Conflict of interest statement

The authors declare no conflict of interest in the current research work

Animal ethics committee permission

The current research work is permitted to be executed according to standards of animal research committee in the Faculty of Veterinary Medicine, Mansoura University

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