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SHORT COMMUNICATION

The potential effects of *Spirulina platensis* (*Arthrospira platensis*) on tissue protection of Nile tilapia (*Oreochromis niloticus*) through estimation of P53 level

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ABSTRACT

The current study was designed to investigate the potential effect of *Spirulina platensis*, *Arthrospira platensis*, (*SP*) on tissue protection of Nile tilapia (*Oreochromis niloticus*) through estimation of P53 level. Five isonitrogenous and isocaloric rations containing graded levels of dried SP 5, 7.5,10, 15, and 20 g/kg diet were fed separately to five equal groups of *O. niloticus* fingerlings, additional control group was assigned for 3 months. Liver samples were separately collected from each group by the end of each month. The expression level of P53 showed a substantial decrease among the treated groups in a time-dependent manner. It is therefore advisable to incorporate *SP* in diets for tissue protection and antioxidant effects in cultured *O. niloticus*.

Introduction

Tilapias are among the easiest and most profitable fish candidate to aqua-farm [1], being omnivorous render it susceptible to utilize cyanobacterial blue-green algae [2]. Using feeds in aquaculture generally increases both cost and productivity and hence there is a vital need to seek effective ingredients that can either partially or totally replace expensive ingredients as protein sources [3].

Spirulina platensis (*SP*), a nutritionally enriched filamentous cyanobacterium, possesses diverse biological and nutritional significance having bio-modulatory and immuno-modulatory functions. [4] Algae gained attention as a possible alternative protein source for cultured fish, particularly in tropical and subtropical regions where algae production is high [5]. *SP* is well known for its anti-oxidant and anti-cancerous properties as well as its ability to amend the carcinogen-damaged DNA [6,7].

The P53 protein is the founding member of a family of proteins that regulate cell cycle progression, differentiation, and apoptosis [8]. The P53 gene product is a DNA sequence-specific transcription factor, which as a homotetramer controls

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the expression of a wide-array of genes through direct binding with response elements [8]. Many studies proved that P53 has regulatory responses to a variety of cellular stresses, including DNA damage, nucleotide depletion, chemotherapeutic drugs and oxidative stress, genotoxic damages, oncogene activation, and hypoxia [9–11]. Activation of P53 can induce several responses, including DNA repair, senescence, differentiation, and inhibition of angiogenesis [12]. The P53 acts biochemically as a transcription factor and biologically as a powerful tumor suppressor. The loss of the protective P53 cellular mechanism may eventually result in cancer progression [12]. The major function for the P53 tumor suppressor is to restrict abnormal or stress-exposed cells before damaged DNA converted to inherited mutation [13]. However, even without extended stress, the DNA is exposed to endogenous damaging reactive oxygen species (ROS), which are the by-products of normal respiration, and important signaling molecules [14].

None of the previous studies explored the potential effects of *SP* as an anti-oxidant agent in *Oreochromis niloticus*. The aim of the present work was therefore to estimate the *in vitro* potential effects of *SP* as a chemoprotective agent in *O. niloticus* through estimation of P53 expression level.

Material and methods

Fish

A total of 2400 *O. niloticus* fries (mean individual initial weight 4 ± 1.0 g) were divided into six equal groups, each consisting of four replicates (100 fry/replicate) in 6 separate earthen ponds. Fish in each replicate were reared in a hapa made of cotton mesh like a cage (3 × 2 × 1 m, each) that was fixed in an earthen pond (for each group, a total of 4 hapas were equally arranged in 4 rows). The fish were fed twice daily on a basal diet of 35% protein at 10% of body weight per day. The feed was placed in plastic trays fitted in the hapas (1 per hapa). The water was partially renewed daily and monitored regularly and maintained at 25 ± 1 °C. The whole experimental work was carried out at the experimental units of The World Fish Center (Abbasa, Sharkia, Egypt) and was approved by ethical committee of Faculty of Veterinary Medicine, Cairo University.

S. platensis (SP)

Pure dried *SP* tablets were obtained from Lake Heath Products Co., Ltd. (Liyang City, Jiangsu Province, China). The tablets were grounded to a powder form before usage.

Rations

A standard commercial ration containing crude protein, crude lipid, vitamins, and minerals that met the basic dietary requirements of *O. niloticus* was prepared as shown in Table 1. The ingredients were mixed mechanically at room temperature by horizontal mixer (Hobarts model D300-T, Troy, OH, USA). *SP* treated diets were prepared by mixing separately a graded concentration of *SP* at 5, 7.5, 10, 15, and 20 g/kg diet. The pellets were then prepared using a pellet-machine (California Pellet Mill, Roskamp Huller Co., California, USA) with 0.5 cm diameter. The pellets were left for 24 h for air-drying at room temperature (26 °C), broken into small pieces, and sieved to obtain the appropriate size. The rations were transferred into plastic bags and stored in a refrigerator at 4 °C until used. The last group was assigned to a control ration and received the standard commercial pellet without any treatment. The required diet was prepared biweekly and stored in a refrigerator (4 °C) for daily use.

Experimental design

Three months feeding study periods were conducted to evaluate the efficacy of *SP* as a chemoprotective agent in cultured *O. niloticus*. The pre-acclimated fish were divided into 6 equal groups. Group 1 was fed on a basal diet (control) and the other groups were dietary supplemented with a single graded concentration of dried *SP* at 5, 7.5, 10, 15, and 20 g kg^{-1} diet fed, respectively. At the end of each month, the P53 expression level relative to control was estimated in liver of experimental groups.

Tissue sampling

By the end of each month; liver specimens were collected from fish representing each treatment separately. Samples were collected in sterile 0.5 ml cryotubes, freeze, and stored at −80 °C until used.

Total RNA extraction and cDNA synthesis

Total RNA isolation was performed using QIAmp RNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer manual. The isolated RNA was used in cDNA synthesis using reverse transcriptase (Fermentas, EU).

Real-time PCR (qPCR)

The reaction mixture consisted of 1 μl cDNA, 0.5 mM of each primer (P53 and GAPDH as internal control), iQ SYBR GREEN PERMIX (BIO-RAD 170–880, USA) in a total volume of 20 μl. PCR amplification and analysis were achieved using BIO-RAD iCycler thermal cycler and the MyiQ real-time PCR detection system. All templates were amplified using the following Light Cycler protocol. The primer for P53 was based on the sequence published in gene bank FJ233106.1 for *O. niloticus*; forward primer; GCATGTGGCTGATGTTGTTC and the reverse one GCAGGATGGTGGTCATCTCT. The fast start polymerase was activated and cDNA denatured by a pre-incubation for 10 min at 95 °C, the template was amplified for 40 cycles of denaturation programed for 20 s at 95 °C, annealing of primers at 60 °C programed for 20 s, and extension at 72 °C programed for 30 s. Fluorescent data were acquired during each extension phase. Each assay includes triplicate samples for each tested cDNAs and no-template negative control [15].

The ΔCT value is calculated by the subtraction of the GAPDH CT from each P53 CT.

- The ΔΔCT value is calculated by subtraction of the control ΔCT from each P53 ΔCT.

Table 1 Composition of the *Oreochromis niloticus* basal diet used throughout the experiment.

Ingredients	Diet (%)	Protein (%)		Metabolic energy (J)	
		Ingredients	Feed	Ingredients	Feed
Fish meal	7.95	0.72	5.76	4000	32,000
Soybean meal	52.8	0.48	25.39	2870	151,823
Ground corn	29.1	0.10	3.17	1240	36,084
Wheat flour	5.00	0.13	0.67	2700	13,500
Vegetable oil	2.00	0.00	0.00	9100	18,200
Cod liver oil	2.00	0.00	0.00	9100	18,200
Di calcium phosphate	1.00	0.00	0.00	0.00	0000
Mineral mix.	0.07	0.00	0.00	0.00	0000
Vitamin mix.	0.05	0.00	0.00	0.00	0000
Total	100	0.00	34.99	0.00	269,807

- The expression relative to control is calculated using the equation $2^{-\Delta\Delta CT}$.

Statistical analysis

A two way ANOVA model followed by post-Hoc test MCT (LSD) was used for data analysis. The computation was executed on SPSS program version 15 and a *P* value less than 5% is considered significant ($p < 0.05$).

Results

The expression level of P53 showed a decline pattern among the treated groups relative to the control group (Table 2). The two way ANOVA analysis based on *F* test showed a *P* value of 0.284 between groups, which is not significant. There were observed differences between groups receiving different concentrations of *SP;* however, it was not sufficient enough to be significant. LSD between time showed a significant difference between first month and third month with a *P* value equal 0.025 (< 0.05) (Table 3).

Discussion

Among the diverse biological activities and nutritional significance of Spirulina, their capability to inhibit carcinogenesis via its anti-oxidant properties has been previously reported [6]. Based on two ways ANOVA statistical analysis test, we found that *SP* could produce a chemoprotective action in *O. niloticus* when supplemented in the ration for at least 2 months regardless the dose administered to fish.

Table 2 Results of P53 expression level relative to control in *Oreochromis niloticus* fed PS for 3 months.

	First month	Second month	Third month
Group 2 (5 g/kg)	1.0	2.3	0.64
Group 3 (7.5 g/kg)	1.1	0.37	0.33
Group 4 (10 g/kg)	1.1	0.31	0.13
Group 5 (15 g/kg)	1.2	0.34	0.11
Group 6 (20 g/kg)	1.1	0.34	0.12

Table 3 Results of statistical analysis of P53 expression level: Mean, Std. Error of groups, and duration of experimental feeding trials of *Oreochromis niloticus* with PS.

Group	Mean	Std. Error
Group 2 (5 g/kg)	1.313	0.280
Group 3 (7.5 g/kg)	0.600	0.280
Group 4 (10 g/kg)	0.513	0.280
Group 5 (15 g/kg)	0.550	0.280
Group 6 (20 g/kg)	0.520	0.280
Month	Mean	Std. Error
1	1.100	0.217
2	0.732	0.217
3	0.266	0.217

Based on sample size for groups = 5 and sample size for months 3.

The findings of the present study are consistent with the growing body evidence indicating that in addition to the P53-dependent transcriptional program, its known influence on apoptosis and cell cycle arrest enhances the expression of key regulators of innate immunity pathways [16]. P53 may extend its protective function by participating in antioxidant defense. Such activity should be at variance to the known pro-oxidant function of some stress-induced P53-responsive genes, which contribute to P53-induced cell death [17]. All these findings suggest that the highly conserved nature of P53 among eukaryotes may rely more on its role in host immunity rather than its functions as a tumor suppressor gene [18].

The functions of the P53 tumor suppressor that restrict proliferation of abnormal cells are activated by stresses presuming that under normal conditions, P53 is dormant. However, P53 might have additional non-restrictive functions addressing physiological stresses, which produce repairable injuries. One of the emerging protective functions of P53 is the enhancement of DNA repair [19]. Taura et al. noticed that in addition to P53 down-regulates intracellular ROS levels (thus reducing probability of genetic alterations), the antioxidant function was not expected as P53 was known as a potent pro-oxidant inducing a set of ROS-generating genes, which contribute to apoptosis. However, the anti-oxidant function of P53 is mediated through a set of antioxidant genes, which are responsive to lower levels of P53 in non-stressed or physiologically-stressed cells [20]. We propose that the antioxidant function of P53 represent an important component of its suppressor activity, which de-

creases probability of genetic alterations and assists the survival and repair of cells with minor cellular injuries [21,22]. Also, the findings of our study can be assisted by the reports proved the P53-dependent enhancement of interferon regulatory factor (IFN) signaling and other genes involved in innate immunity including IRF5, antiviral genes such as ISG15 and double stranded RNA (dsRNA)-activated protein kinase R (PKR), and pro-inflammatory chemokines such as monocyte chemoattractant protein 1 (MCP-1) [23].

Conclusion

We could conclude that the incorporation of dried *SP* was useful to positively improve the health conditions of Nile tilapia through tissue protection and anti-oxidant effects of *SP via estimation of P53 expression level*. It is recommended to supplement Spirulina in the diet of Nile tilapia especially those grow in farms under immunosuppressive/stressful conditions. The supplementation must be for a minimum of 2 months to exert its beneficial effects. Additional researches are needed to study the possible additional desired effects of the blue-green algae in cultured fish.

Conflict of interest

The authors have declared no conflict of interest.

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