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Effect of dietary supplementation with *Spirulina platensis* on Atrazine-induced oxidative stress- mediated hepatic damage and inflammation in the common carp (*Cyprinus carpio* L.)

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ABSTRACT

The present study evaluated the potential modulatory effect(s) of dietary supplementation *with Spirulina platensis* (SP) on Atrazine (ATZ)-induced oxidative stress and inflammation in common carp *(Cyprinus carpio* L.*)*. Common carp was exposed to ATZ (428 μg/L) and SP (1%), either alone or in combination, for 40 days. Subsequently, the treatment groups were evaluated for ATZ-induced oxidative stress-mediated hepatic damage and the potential antioxidant effect(s) of SP supplementation. The results indicated that ATZ exposure led to a significant increase in the oxidative stress as suggested by the increased levels of lipid and DNA oxidative damage markers and the significant decline of antioxidant status biomarkers. Further, a real-time PCR analysis of the liver tissues revealed that the ATZ exposure resulted in the significant modulation of the mRNA expression of cytokines involved in the inflammatory response pathway in the liver, such as Interleukin (IL)−1ß and IL-10. The expression of *IL-1ß* mRNA was up-regulated while that of IL-10 mRNA was down-regulated. The group subjected to supplementation with SP exhibited a significant decrease in ATZ-induced oxidative stress-mediated hepatotoxic and inflammatory responses; however, these did not attain the levels of the control group. Owing to its ability for protecting against ATZ-induced oxidative stress-mediated hepatic damage in carps, SP could be a potentially effective and promising candidate as a feed additive for carps in aquaculture.

1. Introduction

The decline and endangering of freshwater species is closely linked with various anthropogenic activities, for example, the use of herbicides, pesticides, etc. This occurs because unlike in marine systems, the probability of pollutant dilution is rare in freshwater systems, resulting in an increase in the mortality of biological life forms. This can have severe negative implications for biodiversity and the aquaculture industry (Runge et al., 2003).

Atrazine (ATZ) is a commonly used herbicide for controlling broadleaf and grassy weeds in crops fields such as maize and sugarcane. It is used as a pre-emergent herbicide for cereals and for submerged vegetation in stagnant and slow running waters (Goldman, 1994). ATZ is thought to be a potential contaminant in aquatic habitats, where it may gain entry through run-offs from treated fields, spillage, or accidental discharge, as well as frequent torrential rains. Hence, ATZ residues are found in many environments, especially in surface and ground waters (Wightwick and Allinson, 2007). Owing to ATZ's relative persistence in soil, moderate solubility in water, and a half-life, ranging from a few days to months, contamination of surface and ground waters by ATZ is on the ascent in several countries (Kreutz et al., 2012).

Earlier studies on the acute toxicity of ATZ in juvenile rainbow trout (Shelley et al., 2012) and common carp (Khalil et al., 2017) have shown that it adversely influences their immune responses. Further, ATZ has been found to act as an enzyme inhibitor in the neotropical fish, *Prochilodus lineatus*, thereby resulting in impairment of hepatic metabolism and genotoxic damage to various cell types (Santos and Martinez, 2012). Likewise, ATZ treatment was shown to negatively affect acetylcholinesterase activity and mRNA levels in muscles, brain (Xing et al., 2010), kidneys, liver, and gills (Xing et al., 2013) of common carp. AZT has been found to induce oxidative stress (Jin et al., 2010b) and disturb the swimming performance of larval zebrafish (Liu et al., 2016). Consequently, many European and African countries have limited its use (Coady et al., 2005).

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Spirulina platensis (SP) is a filamentous photosynthetic microalga (Ismail et al., 2009). It is a rich source of protein, minerals, vitamins, essential amino acids, essential fatty acids, and pigments, and hence, one of the most commonly used supplements in both human and animal species (Abdelkhalek et al., 2015). Recently, some studies have shown the anti-oxidant, growth-promoting, anti-inflammatory, and immune-modulatory activities of SP (Watanuki et al., 2006). SP is a source of various bioactive compounds such as phycocyanin, ß-carotene, and allophycocyanin, which possess antioxidant and anti-inflammatory activities, sulfated polysaccharides which exhibit anti-viral properties, and sterols, which elicit anti-microbial effects (Wang et al., 2007).

Studies using SP as a supplement and as a partial substitution in the diets have noted that SP improves immune responses in Mekong giant catfish (Tongsiri et al., 2010), and African sharp tooth catfish (Promya and Chitmanat, 2011). Moreover, SP has been reported to improve resistance against infection in the shrimp, *Litopenaeus vannamei* (Chen et al., 2016). Further, SP is accounted for alleviating organ toxicities induced by heavy metals, such as cadmium and lead (Simsek et al., 2009). Owing to its free radical scavenging and potent antioxidant activity, SP administration has been shown to limit the pesticide-induced toxic effects of deltamethrin on *Oreochromis niloticus* (Abdelkhalek et al., 2015). Likewise, SP polysaccharide was found to possess anti-mutagenic and hematopoietic system activation potential when studied in animal model systems (Rehab and Makhlouf, 2012).

Fish can be used as biomarkers of environmental pollution and can assume noteworthy roles in evaluating potential hazard related to pollution in the aquatic environment since they are directly exposed to chemicals either directly from surface run-off waters or indirectly through the food chain of the ecosystem (Lakra and Nagpure, 2009). Common carp (*Cyprinus carpio* L.*)*. is a widely distributed and highly consumed freshwater omnivore fish of economic significance. Additionally, there is a growing body of scientific literature generated using common carp as a model system and focuses on the impacts of contaminants at the molecular, cellular, and systemic levels, suggesting its suitability for the use as a model for determining toxicity and its repair (Oruç and Usta, 2007).

The present study relates to the investigations on the role of SP in mitigating the responses caused due to the effect of ATZ-based pesticides contamination on the liver of common carp (*Cyprinus carpio* L.). Investigations included measurement of the antioxidant status, oxidative stress endpoints, and mRNA gene expression analysis of cytokines associated with the inflammatory response pathway in the liver.

2. Materials and methods

2.1. Tested compounds and diet preparation

ATZ [2-chloro-4-ethylamino-6-isopropyl-amino-s-triazine] (98% purity) was obtained from Sigma-Aldrich Company, St. Louis, USA. ATZ was dissolved and diluted in dimethyl sulfoxide (DMSO; SERVA Electrophoresis GmbH, Heidelberg, Germany). *Spirulina platensis* (Sp), in the form of a blue-green powder, was purchased from EL-Hellowa for Biological Products. SP supplemented diet was constituted by blending SP separately at 1%. The components were blended mechanically followed by the preparation of pellets using a pellet machine then the pellets were air-dried at room temperature (27 °C) for 24 h and stored at 4 °C until used.

2.2. Fish

A total of 72 apparently healthy *Cyprinus carpio* (118 ± 4.2 g) fish were obtained from Abbassa Fish Hatchery, EL-Sharkia Governorate, Egypt. A group of 10 fish was kept in a glass aquarium loaded with 60 L of dechlorinated tap water for two weeks and fed on a basal diet for acclimatization. All the aquaria were kept under similar conditions of water temperature (25 ± 1.02 °C), pH (6.9 ± 0.1), dissolved oxygen (7.4 ± 0.34 mg/L), and ammonia (0.035 ± 0.01 mg/L) with a controlled photoperiod (10 h light: 14 h dark) in the laboratory. The fish were fed twice every day (9:00 a.m. and 3:00 p.m.) at a rate of 3% of their biomass.

2.3. Experimental design and diet

The fish were randomly assigned into four groups, each group having two replicates (9 fish/replicate). The control group was fed on a basal diet and DMSO was directly added to the water. The *Spirulina platensis* group (SP) was fed on a basal diet supplemented with 1% SP (Abdelkhalek et al., 2015). The Atrazine group (ATZ) was exposed to ATZ at a dose of $^{1}/_{5}$ 96-h LC_{50} value (428 µg/L) (the calculated 96-h LC_{50} of ATZ is 2.142 mg/L; Xing et al., 2010). The protective co-treated group (ATZ/SP) was fed on a basal diet supplemented with 1% SP and exposed to ATZ at the same dose as previously mentioned. The water was completely replaced at every 48 h by exchanging the fish to freshly prepared pesticide solutions. Throughout the exposure period (40 days), the fish were monitored for clinical signs, post-mortem lesions, and mortalities. The experimental procedures were endorsed by the Ethics of Animal Use in Research Committee (EAURC) of Cairo University, which are based on the NIH general rules for the Care and Use of Laboratory Animals in scientific investigations.

2.4. Sampling and tissue collection

The blood samples were collected at the end of the experimental period (40 days) from the caudal vessels (n = 3/replicate) to obtain the serum by centrifugation at 3000 rpm for 15 min for biochemical analysis. After sacrificing the experimental and control fish by cervical decapitation, the liver specimens were immediately dissected into three sets: first set (n = 3/replicate) washed with ice-cold phosphate buffered saline (PBS), then homogenized for analysis. The second set (n = 3/replicate) stored at −80 °C until real-time PCR analysis was carried out. The third set (n = 3/replicate) was used to prepare the cells for DNA damage analysis (comet assay) by setting in 1 mL of cold Hank's balanced salt solution containing 20 mM ethylenediaminetetraacetic acid and 10% DMSO and it was minced into fine pieces to acquire cell suspensions.

2.5. Antioxidant biomarkers in hepatic tissue

The activities of superoxide dismutase (SOD) and catalase (CAT) and the level of reduced glutathione (GSH) were measured according to methods described previously (Misra and Fridovich, 1972; Sinha, 1972; Beutler et al., 1963).

2.6. Oxidative stress biomarkers (lipid peroxidation, protein oxidation, and DNA damage) in hepatic tissue

Lipid peroxides (malondialdehyde; MDA) were measured by a colorimetric assay as described previously (Ohkawa et al., 1979). Protein carbonyl (PC), as a marker of protein oxidative damage, was measured using a colorimetric assay kit (Cayman Chemical Company; Ann Arbor, MI, USA) following the protocol of Levine et al. (1994). The comet assay was done following the method described by Singh et al. (1988). Fifty randomly selected cells per slide were investigated. A fluorescence microscope (Zeiss Axiovert L410 Inc., Germany) connected with a digital camera (Olympus Inc., Japan) was used for the imaging of cells. The comets were analyzed by a visual scoring method and computer image analysis was done using the Comet Assay Project Software.

2.7. Serum hepatic injury markers and bilirubin contents

Commercial kits for the quantitative determination of total protein, albumin, total, and direct bilirubin, and the levels of liver enzymes,