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Ameliorative Effect of Lycopene on Haematological Indices of Common Carp *Cyprinus carpio*, Linnaeus, 1758 Exposed to Cypermethrin

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ABSTRACT

The present study was conducted to investigate the effectiveness of lycopene in alleviating the toxicity of cypermethrin (CYP) on haematological parameters in carp, *Cyprinus carpio*. Fish (totally 140 fish) were exposed to sublethal concentrations (0.202 and 0.404 µg/L) of CYP, and lycopene (10 mg per kg of fish weight) was simultaneously administered. At the end of 28 days administration, blood samples were collected and haematological changes (red and white blood cell count, haemoglobin concentration, haematocrit level, and erythrocyte indices: mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration) were determined. According to the data obtained, CYP was determined to lead to negative alterations in the haematological parameters investigated. The administration of lycopene alleviated this effect.

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Introduction

Cypermethrin (CYP) is a synthetic pyrethroid insecticide used to control many pests, such as moth pests attacking cotton, fruit and vegetable crops, including structural pest control, or landscape maintenance. This has resulted in its discharge into the aquatic environment and consequently several laboratory studies have been performed, which have shown that CYP is extremely toxic to fish and aquatic invertebrates at very low concentrations. Fish sensitivity to pyrethroids may be explained by their relatively slow metabolism and elimination of these compounds (David et al., 2003).

The exposure of fish to several types of chemical agents may induce changes in several haematological parameters, which are frequently used to evaluate fish health. Haematology has been widely used for the detection of physiopathological alterations following exposure to different stress conditions. Therefore, haematological techniques are the most common method to determine the sublethal effects of pollutants (Modesto and Martinez, 2010; Kumar et al., 2011; Mişe Yonar et al., 2014).

The carotenoids are a family of fat-soluble pigments that are prevalent in numerous fruits and vegetables. Several studies have investigated the potential of carotenoids to ameliorate oxidative stress. Lycopene, which is a naturally occurring carotenoid that is present in tomatoes and tomato products, has attracted considerable attention as a potential chemopreventive agent. Recently, interest in lycopene has increased as a result of its highly efficient antioxidant scavenging activity against singlet-oxygen and free radicals. Thus, lycopene may prevent oxidative damage, toxicity, and disease (Yonar and Sakin, 2011; Yonar, 2013).

The ameliorative effect of lycopene against CYP-induced changes in the haematological indices of fish have not so far been studied. Therefore, the objectives of the study were to evaluate whether CYP induces changes in the haematological parameters of carp and to evaluate the role of lycopene in alleviating the negative effects of CYP.

Materials and Methods

Chemicals and Fish Samples

The chemicals used in this study were obtained from Sigma–Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany). The commercial product CYP (Trade name: Polytrin 200 EC, Novartis) available at a concentration of 20% was used in this study. Lycopene (10% fat-soluble, Redivivo TM, Code 7803) was purchased from DSM Nutritional Products (Istanbul, Turkey). Pure corn oil was used to dissolve lycopene, which is insoluble in water (Boileau et al., 2002; Yonar, 2013).

Carp (*Cyprinus carpio*) were obtained from local fish culture pools of the State Hydraulic Works, Elazig, Turkey (Elazig, Turkey). The fish (72.59±13.30 g and 14.81±0.67) were transported to the laboratory and acclimatised in stock tanks (540 L capacity; 80x75x90 cm) to laboratory conditions for 2 weeks at 22±2°C with a pH of 7.4±0.2, a dissolved oxygen content of 7.6±0.5 mg/L, and a 12-h light/12-h dark photoperiod. During this period, the fish were supplied commercial fish food twice daily. The use of fish and the experimental protocol were approved by the Animal Experimentation Ethics Committee of the Firat University (FUAEEC, Protocol No:24/2011 (Elazig, Turkey).

Carp is the most produced fish species in the world. It is a species that is both cultivated and naturally distributed in our country. It can be adapted easily to the laboratory environment. This species was used in the present study due to these properties.

Feed Preparation

Lycopene was suspended in corn oil. A commercial basal diet was crushed and divided into two parts. The first part was mixed with 10 mg lycopene for per kg fish weight, the second part was mixed with corn oil. The diet was reformed into pellets, spread to dry and stored at + 4°C for the feeding experiment. Re-made pellets were given to the fish manually at a rate of approximately 2% fish body weight per day.

Experimental Setup

After two weeks of acclimation, the fish were randomly divided into seven groups that each contained 10 fish. The first group was held in tap water as a control group. Fish in group 2 received corn oil orally for 28 days. Fish in group 3 received lycopene orally for 28 days. Fish in group 4 were exposed to 0.202 µg/L CYP in their environment for 28 days. Fish in group 5 were exposed to 0.202 µg/L CYP, while lycopene was simultaneously administered for 28 days. Fish in group 6 were exposed to 0.404 µg/L CYP for 28 days. Fish in group 7 were exposed to 0.404 µg/L CYP while lycopene was simultaneously administered for 28 days. The entire experiment was repeated three independent times; each replicate for each group contained 10 fish, for a total of 140 fish.

Sublethal concentrations were selected based on 96-h LC_{50} value for *C. carpio*. According to Aydın et al. (2005), the 96-h LC_{50} of CYP for *C. carpio* was 0.809 µg/L. Fish were exposed to 0.202 µg/L (approximately 1/4 of 96-h LC_{50}) and 0.404 µg/L (approximately 1/2 of 96-h LC_{50}) of CYP for 28 days. Experimental aquaria were aerated and test media were replaced every day. No fish mortality occurred during these exposures.

Collection of Samples and Haematological Analyses

At the end of the experiment, fish were anaesthetised with benzocaine (25 mg L^{-1}) and blood samples were drawn from the caudal peduncle using a medical syringe with EDTA as an anticoagulant. The blood was used immediately for haematological analysis. The red blood cell (RBC) and white blood cell (WBC) counts were made using a haemocytometer and Natt and Herrick (1952) solution. The haemoglobin concentration (Hb) was determined with Drabkin's reagent read at 540 nm (Drabkin 1946), and the haematocrit (Ht) was determined by a microhaematocrit centrifugation technique. The erythrocyte indices [mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC)] were calculated by standard formulas with the data of the Ht, RBC and Hb (Saglam et al. 2003, Saglam and Yonar 2009).

Statistical Analyses

The results are expressed as the means ± standard error. The statistical significance of the differences between the data obtained from the control and that obtained from the experimental groups was analysed via analysis of variance (one-way ANOVA) and Duncan's post-hoc test using the SPSS 21 computer program (SPSS). P-values <0.05 were considered to be statistically significant.

Results

The effects of CYP, lycopene, and their combination on the haematological parameters of the fish are provided in Table 1. The results showed that CYP alone causes a significant decrease in the RBC count, the Ht level, the Hb concentration, and the erythrocyte indices. The WBC count increased in the groups that exposed to CYP alone. Simultaneous treatment with lycopene provided a marked normalisation of the haematological parameters when compared with the CYP groups. The RBC and WBC counts and the Ht levels of the group that received lycopene alone differed significantly from those of the control group.

Discussion

Haematological parameters of fish can provide important information about the internal environment of the organism. The evaluation of haematological characteristics in fish has become an important means of understanding normal and pathological processes and toxicological impacts (Li et al. 2011, Dotta et al. 2014). Haematological parameters, such as the RBC counts, Hb concentrations, Ht levels, and the erythrocyte indices (MCV, MCH and MCHC), are widely used to evaluate the toxic stress that is induced by environmental contaminants (Saravanan et al. 2011). In this study, the RBC, Ht and Hb level, and the erythrocyte indices were decreased significantly in the CYP treated fish. Masud and Singh (2013) also reported that significantly lower values of red blood cells and haemoglobin level are found in carp exposed to CYP for seven days. Similarly, Atamanalp et al. (2002) found a significant decrease in the Hb and Ht levels in freshwater fish, *Capoeta capoeta* exposed to CYP. Decreases in the RBC count and the Ht and Hb levels may be an indicator of anaemia due to the

inhibition of erythropoiesis, haemosynthesis, or osmoregulatory dysfunction or to an increased rate of erythrocyte destruction in the hematopoietic organ (Vani et al. 2011; Mişe Yonar 2013; Mişe Yonar et al. 2014). In addition, decreases in the MCV, MCH, and MCHC values indicate microcytic hypochromic anaemia (Prusty et al. 2011). Unlike mammals, the hematopoietic system of fish is mainly located in the interstitium of the kidney. So, a reduction in the haematological parameters may be attributed to the malfunctioning of the hematopoietic system caused by morphological alterations in renal interstitium (Li et al. 2011). However, the haematological values of the groups treated with lycopene were similar to those of the control group and were significantly different from those of the groups that were exposed only to CYP. The findings of our study suggest that lycopene may be helpful in reducing the harmful effects of CYP by maintaining optimal haematological values.

Table 1 Effect of CYP, lycopene and their combination on some haematological parameters in the control and experimental groups.

G*	RBC ($x10^6$)	WBC ($x10^3$)	Ht (%)	Hb (g/dL)	MCV (μm^3)	MCH (pg)	MCHC (%)
1	1.42 ± 0.11^{d}	30.58 ± 2.74^{a}	31.70 ± 3.20^{b}	6.98 ± 1.20^{b}	224.25 ± 22.41^{b}	48.54 ± 4.33^{b}	22.18 ± 3.25^{b}
2	1.41 ± 0.14^{d}	31.04 ± 3.28^{a}	30.85 ± 2.95^{b}	6.65 ± 0.72^{b}	220.74 ± 29.32^{b}	47.63 ± 5.24^{b}	21.59 ± 3.84^{b}
3	1.47 ± 0.10^{e}	36.77 ± 4.83^{b}	35.24 ± 3.16^{c}	7.02 ± 0.99^{b}	242.19 ± 35.74^{c}	47.75 ± 4.19^{b}	20.94 ± 4.23^{b}
4	1.18 ± 0.08^{b}	35.94 ± 3.10^{b}	22.06 ± 3.21^{a}	4.21 ± 0.52^{a}	185.91 ± 19.85^{a}	34.79 ± 5.13^{a}	17.08 ± 3.41^{a}
5	1.36 ± 0.12^{c}	31.22 ± 2.56^{a}	29.45 ± 3.70^{b}	6.53 ± 0.76^{b}	217.44 ± 21.08^{b}	48.14 ± 6.22^{b}	22.31 ± 4.69^{b}
6	1.06 ± 0.09^{a}	37.45 ± 4.14^{b}	21.19 ± 2.45^{a}	4.03 ± 0.83^{a}	196.05 ± 37.73^{a}	38.88 ± 3.59^{a}	16.01 ± 2.87^{a}
7	1.34 ± 0.11^{c}	31.59 ± 3.12^{a}	29.20 ± 3.12^{b}	6.61 ± 1.04^{b}	218.91 ± 13.95^{b}	47.32 ± 5.61^{b}	22.13 ± 3.56^{b}

RBC: red blood cell (erythrocyte) counts, WBC: white blood cell (leukocyte) counts, Ht: haematocrit, Hb: haemoglobin concentration, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, Different superscripts in the same column indicate significant differences between groups ($P<0.05$), *: Group 1, control; Group 2, corn oil; Group 3, lycopene (10 mg/kg fish/day); Group 4, CYP (0.202 µg/L); Group 5, CYP (0.202 µg/L) plus lycopene (10 mg/kg fish/day); Group 6, CYP (0.404 µg/L); Group 7, CYP (0.404 µg/L) plus lycopene (10 mg/kg fish/day).

Leucocytes play an important role in nonspecific or innate immunity, and the leucocyte count/activity can indicate the health status of a fish (Secombes 1996). The results of the present study indicate that the WBC count was significantly higher in the CYP treated fish. Similar changed the count of WBC in common carp have also been reported by other authors. Ramesh and Saravanan (2008) reported an increase in the WBC count of carp after chlorpyrifos exposure. Yonar et al. (2012) reported that chlorpyrifos significantly increased the leucocyte count in *C. carpio*. Similarly, Masud and Singh (2013) reported that significantly higher values of white blood cells occurred in *C. carpio* after exposure to CYP for seven days. This increase in the WBC count could be the result of the activation of the immune system in the presence of a contaminant, which may in turn be an adaptive response of the organism, resulting in a more effective immune defence response (Modesto and Martinez, 2010). However, the WBC count of the groups treated with lycopene were similar to the control group and significantly different from the groups that were exposed to only CYP.

In conclusion, the administration of CYP to carp caused significant changes in certain haematological parameters (the red blood cell and white blood cell counts, the haematocrit level, the haemoglobin concentration, and the erythrocyte indices). The use of lycopene was ascertained to alleviate the harmful effects of CYP in the mentioned parameters.

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