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Original Research

Effect of Tunceli garlic on some immunological parameters in *Cyprinus carpio* exposed to chlorpyrifos

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Abstract: In this study, the effect of Tunceli garlic (*Allium tuncelianium*) on hemoglobin (Hb) level, oxidative radical production of neutrophils (Nitoblue tetrazolium assay-NBT activity) and total immunoglobulin (TI) content in carp (*Cyprinus carpio*) exposed to chlorpyrifos (CPF) was investigated. The 96 hour LC_{50} value of CPF on *C. carpio* was calculated to as 0.230 mg/L. The fishes were exposed to sublethal concentration of chlorpyrifos (1/8 of LC_{50} value: 0.029 mg/L), and Tunceli garlic (20 and 40 g/kg diet) was simultaneously administered for 21 days. Blood samples were taken from the fishes at 7, 14 and 21 days and analysed to determine the Hb levels, the NBT activity and the TI content. There was a significant decrease in the Hb level, the NBT activity and the TI content of CPF-treated fish. However, Tunceli garlic reversed the Hb level, the NBT activity and the TI content. In conclusion, this study demonstrated that CPF had a negative effect on the immunological values of the fish. The simultaneous administration of Tunceli garlic was neutralised CPF-induced toxicity.

Key words: Chlorpyrifos; *Cyprinus carpio*; Hematology; Immunology.

Introduction

Aquatic environments are important biotopes that should be controlled sensitively because of contamination resulting from the industrial, agricultural and anthropogenic activities (1). Organophosphate (OP) pesticides are a significant insecticide class and are extremely toxic to fish (2-7). They show their toxic effects as "cholinesterase inhibitors", so they inhibit acetylcholineasterase (AChE), which hydrolyze acetylcholine (ACh) at muscle-nerve junctions and cholinergic synapses (8). Chlorpyrifos (CPF) is an effective OP pesticide that has a wide use in the world to control various pests in agricultural areas and animal farms (9). CPF, one of the most frequently used insecticides in OPs, is relatively more toxic to fish than organochlorinated pesticides (10). This insecticide has been shown adversely affects immunity system of fish in previous studies (11, 12).

Garlic (*Allium sativum*) is known as an important medicinal plant which has a wide spectrum of effects (13). Tunceli garlic (*Allium tuncelianum*) is an endemic plant species found only in Tunceli province, which especially in the foothills of Munzur mountains Ovacık, Pülümür, Hozat and Pertek districts (14). *A. tuncelianum* has single-cloved bulbs and has small formations like small bulbs, and it can also produce fertile flowers and seeds which with this feature is different from other garlics (15). There are various studies on investigate to effects in the fish of *A. sativum*. It has been reported pharmacological activities of *A. sativum* such as antibacterial (16), antiparaziter (17), antioxidant (18), immunostimulant (19) in previous studies. Seker and Akkan (20) reported Tunceli garlic supplementation was significantly increased some haematological and immunological parameters in fish. The protective role that Tunceli garlic has against CPF-induced changes in immunological parameters of fish have not been studied. Therefore, this present study was to evaluate the possible protective or ameliorative effects of Tunceli garlic on CPF induced some immunological parameters in carp.

Materials and Methods

Ethics statement

All procedures used in the present study were approved by the Animal Experimentation Ethics Committee of Firat University (FUAEEC, Elazig, Turkey) (Protocol number: 2016/36, 126).

Chemicals and fish

The insecticide used for the experiments was a commercial formulation of Korban 4 (480 g/L chlorpyrifos, O,O-diethyl-O-(3,5,6-trichlor-2-pyridyl) phosphorothioate) obtained from a commercial manufacturer. All other chemicals were purchased from Sigma–Aldrich Chemical (St Louis, MO, USA). Carps (*C. carpio*) (102.2 ± 2.64 g) was supplied live from local fish ponds (Elazığ / Turkey) and brought to the laboratory. The fish were kept in ventilated tanks for one month before their experimental work and were adapted at 16.5 ± 1.8 ℃, pH 8.75 ± 0.63, with a dissolved oxygen content of 8.1 ± 0.2 mg/L and a 12:12 light:dark photoperiod. During the adaptation period, fish were fed twice daily with the

commercial fish feed.

Preparation of feed containing Tunceli garlic

Tunceli garlic (*A. tuncelianium*) was obtained from the local people of Tunceli province Ovacık during the harvest period. Garlic peeled and cut into small and thin pieces and dried. The dried and powdered garlic was added to the diets at 20 g/kg (G1) and 40 g/kg diet (G2). The feed was converted into pellets and dried again and stored at + 4 ° C until the experiments started. The remade pellets were administered orally to the fish at a rate of approximately 2% fish body weight per day.

Experimental design

The concentration of CPF chosen for this study was selected on the basis of the 96-h LC50 with confidence limits ($p < 0.05$) that were estimated by using a probit analysis program. After one-month adaptation period, the 96-hour LC_{50} value on *C. carpio* of CPF was determined, and this value is calculated as 0.230 mg/L. Then fishes were divided into 6 groups each containing 30 fish. The study was carried out in 80 L (80x40x25cm) glass aquariums for 21 days. The groups are as follows:

- Group 1 (C), control group, was kept in water without CPF and fed on diet without Tunceli garlic.
- Group 2 (CPF), was applied to sublethal concentration of CPF (1/8 of LC_{50} value: 0,029 mg/L) and fed diet without Tunceli garlic.
- Group 3 (G1), was kept in water without CPF and fed with 20g/kg diet containing feed Tunceli garlic.
- Group 4 (G2), was kept in water without CPF and fed with 40g/kg diet Tunceli garlic containing feed.
- Group 5 (CPF+G1), was applied to sublethal concentration of CPF (1/8 of LC_{50} value: 0,029 mg/L) and fed with Tunceli garlic (20g/kg diet) containing feed.
- Group 6 (CPF+ G2), was applied to sublethal concentration of CPF (1/8 of LC_{50} value: 0,029 mg/L) and fed with Tunceli garlic (40g/kg diet) containing feed.

Sample collection and preparation

On the 7, 14 and 21th days of the study, 10 fish were randomly selected from all groups. The tail fins of fishes anesthetized with bezocain were cut and blood samples were taken to anticuagulant (K_3-EDTA) tubes from caudal vein. The Hb level and the NBT activity were immediately detected in the blood samples taken. The remaining blood samples were centrifuged at 3500 rpm for 15 minutes at 4˚ C to remove plasma. Plasma samples were used to determine the TI contents.

Immunological analysis

The Hb level was determined according to the method described by Drabkin (21), the NBT activity was measured according to Siwicki et al. (22). The TI content was determined with method described by Siwicki and Anderson (23).

Statistical analysis

Statistical analysis of the data obtained at the trial was made using the SPSS 24.0 statistical program. The results are given as median ± standard error. The LC_{50} value of CPF in carp was calculated using Probit analysis. The data obtained from the control and experimental groups were tested by two-way analysis of variance (Oneway-ANOVA). Results were considered statistically significant at $p < 0.05$.

Results

The changes in the Hb level of the control and experimental group fishes are given in the Table 1. The Hb levels of the groups given Tunceli garlic at 20g/kg diet and 40g/kg diet doses were similar to the control group during the study period ($p > 0.05$). It was found that the Hb level of only CPF-treated fish was statistically lower than the control group on all days of the test ($p < 0.05$). The Hb levels of fish exposed to CPF and fed with garlic feeds increased significantly compared to the group only CPF-treated ($p < 0.05$).

The changes in NBT activity of fish in the control and experimental group are given in the Table 2. The NBT activity of fish fed with garlic 20g/kg diet was higher ($p < 0.05$) compared to the control group on the 21th day of experiment, while this activity was similar to the control group on other days. Alone 40g/kg diet garlic administration resulted in increased the NBT activity during the study period, when compared to the control group. A significant decrease in NBT activity occurred in the fish treated with CPF alone. The NBT activity in CPF-administered and garlic-treated groups was higher than in the CPF-treated group alone.

The changes in TI content of fishes in the control and experimental group are given in the Table 3. The TI content of fish given 20g/kg diet garlic is similar to that of the control group during the experiment. The TI content of fish given 40g/kg diet garlic is similar to control at 14th and 21th days. The TI content of fish exposed to CPF decreased when compared with the control group on all days of the experiment. The TI content in the groups that were treated with garlic are decreased those of the control group, however significantly higher than

Table 1. The Hb level in the control and experimental groups (median ± standard error, g/100 ml).

Groups	Days		
	7th	14th	21th
C	$9.70 \pm 1.31^{a;C}$	$9.96 \pm 1.39^{a;C}$	$9.84 \pm 1.40^{a;C}$
G1	$9.64 \pm 2.35^{a;C}$	$9.73 \pm 2.00^{a;C}$	$9.49 \pm 0.90^{a;C}$
G2	$9.90 \pm 1.80^{a;C}$	$9.57 \pm 2.14^{a;C}$	$9.69 \pm 1.42^{a;C}$
CPF	$7.34 \pm 1.44^{c;A}$	$6.19 \pm 1.09^{b;A}$	$5.00 \pm 0.76^{a;A}$
CPF+G1	$8.46 \pm 1.32^{a;B}$	$8.68 \pm 1.29^{a;B}$	$8.70 \pm 1.91^{a;B}$
CPF+G2	$9.47 \pm 1.74^{b;C}$	$8.91 \pm 1.44^{a;B}$	$8.81 \pm 1.62^{a;B}$

^{a,b,c} The difference between the values indicated by different upper symbols on the same line is statistically significant ($p < 0,05$). ^{A,B,C,D} The difference between the values indicated by different top symbols in the same column is statistically significant ($p < 0,05$).

Table 2. The NBT activity in the control and experimental groups (median ± standard error, mg/ml).

Groups	Days		
	7th	14th	21th
C	$0.48 \pm 0.05^{a;D}$	$0.46 \pm 0.04^{a;C}$	$0.47 \pm 0.04^{a;C}$
G1	$0.50 \pm 0.06^{a;D}$	$0.48 \pm 0.05^{a;C}$	$0.54 \pm 0.04^{b;D}$
G2	$0.56 \pm 0.04^{b;E}$	$0.53 \pm 0.04^{a;D}$	$0.60 \pm 0.04^{c;E}$
CPF	$0.11 \pm 0.02^{a;A}$	$0.23 \pm 0.04^{b;A}$	$0.33 \pm 0.04^{c;A}$
CPF+G1	$0.32 \pm 0.05^{a;B}$	$0.40 \pm 0.05^{b;B}$	$0.46 \pm 0.03^{c;C}$
CPF+G2	$0.44 \pm 0.07^{a;C}$	$0.42 \pm 0.05^{a;B}$	$0.43 \pm 0.04^{a;B}$

^{a,b,c} The difference between the values indicated by different upper symbols on the same line is statistically significant ($p < 0,05$). ^{A,B,C,D} The difference between the values indicated by different top symbols in the same column is statistically significant ($p < 0,05$).

Table 3. The TI content in the control and experimental groups (median ± standard error, mg/ml).

Groups	Days		
	7th	14th	21th
C	$13.21 \pm 0.74^{a,D}$	$12.63 \pm 0.58^{a;C}$	$12.80 \pm 0.90^{a;C}$
G1	$13.56 \pm 0.56^{a;D}$	$12.52 \pm 1.08^{b;C}$	$12.31 \pm 0.46^{b;B,C}$
G2	$12.50 \pm 0.47^{a;C}$	$12.86 \pm 0.46^{a;C}$	$12.53 \pm 0.34^{a;B,C}$
CPF	$9.45 \pm 0.67^{a;A}$	$9.48 \pm 0.44^{a;A}$	$9.60 \pm 1.60^{a;A}$
CPF+G1	$10.71 \pm 0.63^{a;B}$	$11.37 \pm 0.76^{b;B}$	$11.54 \pm 0.65^{b;B}$
CPF+G2	$12.32 \pm 0.26^{a;C}$	$11.21 \pm 1.19^{b;B}$	$11.92 \pm 1.47^{a;B}$

^{a,b,c} The difference between the values indicated by different upper symbols on the same line is statistically significant ($p < 0,05$). ^{A,B,C,D} The difference between the values indicated by different top symbols in the same column is statistically significant ($p < 0,05$).

the values for the group that received CPF alone.

Discussion

Haematological variables are important indicators of the health status of fish (24-27). The Hb value can be used as the secondary response of the organism to pollutants and irritants (28). In this study, the Hb level of the CPF-treat alone was found to be lower than the control group on all days of the experiment. This result is similar to the findings obtained by Yonar et al. (29) and Ural (30). They reported that CPF alone caused a significant decrease in the Hb levels of *C. carpio*. The cause of this reduction may be due to the damage caused by CPF in hematopoietic organs. However, the Hb levels of the groups treated with garlic were similar to the control group and significantly different from the groups that were exposed to only CPF. The obtained data suggests that Tunceli garlic may help alleviate the harmful effect of CPF by maintaining optimal the Hb levels.

The NBT activity is used to detect phagocyte activity as an indicator of the immune response (particularly neutrophils and monocytes) (31,32). In this present study, the NBT activity was significantly decreased in the groups CPF-treat alone, when compared to the control group. Mişe Yonar (33) showed that malathion alone causes a significant decrease in the NBT activity. In addition, Kaya et al. (34) found a significant decrease in the NBT activity of carp exposed to OP insecticide fosalon. The results of this study are similar to the results of studies with other OP pesticides. Although, the NBT activity of the groups treated with garlic was significantly different from the group that was exposed to only CPF-treated.

The immunoglobulins are a significant element of specific defence system (35). Li et al. (36) and Banaee et al. (37), reported a decrease in the IgM content of *C. carpio* exposed to CPF. The results of this study indicated that exposure to CPF decreased the TI content of *C. carpio,* corroborating previous studies. Garlic is rich in sulfur-containing amino acids and other compounds that are said to enhance the effectiveness of the immunological system (38) and its specific content of organosulfur compounds are thought to affect the immune system (39). This study finding indicated that CPF alone causes a significant decrease in the TI content and simultaneous treatment with garlic provided a marked normalisation of the TI content.

To summarise, the present study demonstrated that CPF has a harmful effect on the immunological profile. Hovewer, treatment with Tunceli garlic significantly prevents the toxic effects of CPF.

Interest conflict

The authors are responsible for reporting of all financial and personal relationships that might bias their work.

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