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Original Research

Antioxidant effect of curcumin against exposure to malathion in *Cyprinus carpio*

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Abstract: The aim of this study was to determine protective effects of curcumin on oxidant/antioxidant parameters in *Cyprinus carpio* exposed to malathion. The fish were exposed to two sublethal concentrations of malathion (0.5 and 1 mg/L), and curcumin (100 mg per kg of fish weight) was simultaneously administered for 14 days. Malondialdehyde level and superoxide dismutase, catalase, glutathione peroxidase, and glutathione-S-transferase activities were monitored in liver, kidney and gills, which were collected at the end of the experiment. The results revealed a significant increase in the malondialdehyde levels of the groups that were exposed to malathion. Also, malathion exposure caused a significant increase in superoxide dismutase, catalase, and glutathione-S-transferase activities and a significant decrease in glutathione peroxidase activity. Treatment with curcumin attenuated the malathion-induced oxidative stress by significantly decreasing the levels of malondialdehyde in the tissues. In addition, curcumin reversed the superoxide dismutase, catalase, glutathione peroxidase, and glutathione-S-transferase activities. In conclusion, this study demonstrated that malathion caused oxidative stress and negative alterations on the antioxidant enzyme activities of the fish. However, this toxic effect was neutralised by the administration of curcumin.

Key words: Antioxidants; Curcumin; Fish; Malathion; Oxidative stress.

Introduction

Among all kinds of aquatic organisms, fish have been widely used as models to evaluate the quality of aquatic ecosystems and the potential utility of bioindicators for monitoring the health of the organisms because they are quite sensitive to the presence of chemicals in water. Biomarkers are known to be useful tools for measuring environmental exposure of fish to contaminants in laboratory and field studies (1,2).

In aerobic cells, reactive oxygen species (ROS) are free radicals and/or oxygen derivatives produced during normal cellular metabolism, particularly as a result of oxidative metabolism at mitochondrial membranes. At low concentrations, the ROS may be beneficial or even indispensable in processes such as defences against microorganisms, contributing to phagocytic bactericidal activity. In contrast, high doses and/or inadequate removal of these intermediates might be detrimental to the cell, leading to a state called oxidative stress. Oxidative stress cause muscle degradation, impairment of the nervous system, haemolysis, malfunctions of the cellular metabolism and finally cell death (1,3). However, harmful effects of free radicals are neutralized by an antioxidant defence system. As in higher vertebrates, fish also possess two major antioxidant defences, both the nonenzymatic system (glutathione, ascorbate, vitamin E, b-carotene and proteins located in the cytosol and membranes or present in the extra cellular fluid) and enzymatic system (superoxide dismutase, SOD; catalase, CAT; glutathione peroxidase, GSH-Px; glutathione reductase ,GR; glutathione-S-transferase, GST), to protect the cells from damage of ROS (3,4). The SOD is a group of metalloenzymes that are crucial antioxidants. This group of enzymes constitutes the primary defence system against the toxic effects of superoxide radicals (O_2^-) in aerobic organisms (5-7). The CAT is an enzyme located in peroxisomes that facilitates the removal of hydrogen peroxide (H_2O_2), which is metabolised to molecular oxygen and water (8-10). The GSH-Px catalyses the reduction of both H_2O_2 and lipid peroxides (11,12). The GST is multifunctional dimeric enzymes that are involved in detoxification of endogenous (intracellular metabolites) and exogenous substances (drugs, pesticides, and other pollutants) (13,14). These enzymes may be good molecular bioindicators for contaminant-mediated oxidative stress and may also indicate the magnitude of response in populations chronically exposed to contaminants such as pesticide and other xenobiotics (1). They play significant roles in vivo due to their antioxidant function, and their elevated expression and activity are indicative of oxidative stress (15).

Organophosphate compounds are an important insecticide class and are very highly toxic to fish (16). Malathion (O,O-dimethyl-S-1,2-bis ethoxy carbonyl ethyl phosphorodithioate) is a nonsystemic, wide-spectrum pesticide in the organophosphate chemical family and is widely used throughout the world. Several negative effects have been reported as a result of malathion exposure. Negative effects on fish include oxidative damage (4,17,18), changes in growth parameters, haematological properties, swimming ability, and the depletion of some biochemical parameters (glycogen, cholesterol, and total protein content) (17,19,20).

Curcumin, also known as diferuloymethane, is a yellow pigment derived from plant *Curcuma longa* (21).

The powdered rhizome of this plant, called turmeric, has been used in traditional Chinese and Hindu medicine for centuries in the treatment of different diseases (22). Curcumin further exhibits a wide range of pharmacological effects, including antiinflammatory, anticarcinogenic, antitumor, hypocholesterolemic and antiinfection activities (23). As a potential antioxidant, curcumin has been shown to display antioxidative activity, scavenging of superoxide anions and nitric oxide radicals, an inhibition of lipid peroxidation and lipoxygenase/cycloxygenase activity. The strong antioxidant activity of curcumin makes it an interesting candidate for use in counteracting oxidative stress-induced damage (24).

There is no report in the literature on the protective effect of curcumin against malathion-induced oxidative stress in the fish. Therefore the present study was carried out to evaluate the antioxidant and protective effect of curcumin on malathion-induced toxicity in carp.

Materials and Methods

Insecticide

Commercial formulation of malathion (190 g/L malathion, S-1,2 bis (ethoxycarbonyl) ethyl-O,O dimethyl phosphorodithioate) was used in the present work and was purchased from a commercial manufacturer (Safa agriculture companies, Konya, Turkey). Malathion was dissolved in tap water to generate stock solutions. These solutions were then further diluted to obtain the experimental concentrations in the aquariums.

Chemicals

Curcumin (CAS No: 458-37-7) and all the other chemicals were supplied by Sigma-Aldrich Chemical Co. and Merck.

Fish

The test fish, carp (*Cyprinus carpio carpio*) were obtained from local fish culture pools (Elazig, Turkey); average weights of the fish were 52.33 ± 4.20 g. The fish were transported to the laboratory and held in 100 L aquaria containing tap water at 22 ± 1 °C and pH 7.2 until the time of the experiment. Photoperiod was a 12:12 light–dark cycle. The fish were allowed to acclimate to laboratory conditions for a month prior to the start of experimentation. During the acclimation period the fish were fed daily with pelleted commercial food. Guidelines on the care and use of fish in research and testing from the Animal Experimentation Ethics Committee of Firat University (FUAEEC) (Elazig, Turkey) were followed.

Feed preparation

A commercial basal diet was crushed and mixed with 100 mg of curcumin per kg of fish weight. The diet was reformed into pellets, spread to dry, and stored at +4 °C for the feeding experiment. The remade pellets were administered orally to the fish at a rate of approximately 2% fish body weight per day.

Experimental design

The fish were divided into six groups. The first group served as the control group, whereas Group 2 was maintained in tap water and received a diet that contained curcumin for 14 days. The fish in Group 3 were exposed to 0.5 mg/L malathion for 14 days and received a commercial basal diet. The fish in Group 4 were exposed to 0.5 mg/L malathion with the simultaneous administration of curcumin for 14 days. The fish in Group 5 were exposed to 1 mg/L malathion for 14 days and received a commercial basal diet. The fish in Group 6 were exposed to 1 mg/L malathion with the simultaneous administration of curcumin for 14 days.

The entire experiment was independently repeated two times, and each replicate of each group contained eight fish for a total of 96 fish. The sublethal concentrations were chosen according to the malathion 96-h LC50 value previously determined for *C.carpio* (2.10 mg/L) (25). The fish were exposed to 0.5 mg/L (approximately 1/4 of the 96-h LC_{50}) and 1 mg/L (approximately 1/2 of the 96-h LC_{50}) malathion for 14 days. Test media was renewed daily in order to maintain constant concentration of malathion after removal of the same volume of water. No fish mortality occurred during these exposures.

Collection and preparation for analysis of tissues

At the end of the experiment, fish were anaesthetized with benzocaine (25 mg/L water). Then were killed by decapitation and tissue samples (liver, kidney, and gill) were collected from the individual fish. The liver, kidney, and gill washed in ice-cold physiological saline, and stored at -80 °C until the biochemical assays, which were performed within one month after extraction (26,27).

The tissue was homogenised in a Teflon-glass homogeniser in buffer containing 1.15% potassium chloride (KCl) at a 1:10 (w/v) ratio to the whole homogenate. The homogenate was centrifuged (18,000 × g, 4°C, 30 min) to obtain supernatant fraction for the determination of the malondialdehyde (MDA) levels and the SOD, CAT, GSH-Px, and GST activities (28,29).

Measurement of oxidative stress and antioxidant parameters

The method described by Placer et al. (30) was used to determine the MDA levels in all tissues. The measurements were performed in accordance with the method described by Sun et al. (31) for determination of the tissue SOD activity. The CAT activity measurements were performed in accordance with the method described by Aebi (8). The GSH-Px activity measurements were performed in compliance with the method described by Beutler (32). The GST activity was determined by the method of Habig et al. (33). The protein levels in the tissues were determined by the method described by Lowry et al. (34).

Statistical analysis

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

Fish behavior during the experiment

The control and experimental fish showed normal feeding behaviour during the experiment. Furthermore, there were no signs of respiratory distress such as rapid ventilation, increased rate of gill cover movements, or floating at the surface of water. There were no mortalities in the groups during the experiment.

Changes in oxidative stress

The group that received curcumin alone presented statistically significant inhibition in the liver and kidney MDA levels compared with those of the control group. The level of MDA was significantly increased in the liver, kidney, and gill samples of the groups treated only with malathion. Simultaneous treatment with curcumin resulted in a decrease in the tissue MDA levels when compared with the malathion treated groups, but still was more than that of the control. ($p < 0.05$, Tables 1, 2, and 3).

Changes in antioxidant parameters

The group that was administered curcumin alone exhibited statistically significant increases in the liver GSH-Px and GST, the kidney GSH-Px and the gill SOD activities when compared with the control group ($p < 0.05$, Tables 1, 2, and 3).

Significant increases in the activities of SOD, CAT, and GST were observed in the malathion treated fish when compared to non-treated ones. Treatment with curcumin provided a marked normalisation of the tissue SOD, CAT, and GST activities when compared with the malathion groups ($p < 0.05$, Tables 1, 2, and 3).

In the malathion treated groups, significant decrease in the GSH-Px activity was observed in all the analyzed tissues when compared to the control group. However, in the groups that received malathion plus curcumin, the activity of GSH-Px were ascertained to have drawn closer to those of the control group ($p < 0.05$, Tables 1, 2, and 3).

Discussion

In the present study, significant difference was determined in the liver and kidney MDA levels of the group that was administered curcumin alone, in comparison to the control group. Our results are in agreement with Mişe Yonar et al. (35), who have reported curcumin (10, 20 and 40 mg curcumin kg^{-1} diet) to cause decrease in liver, kidney, and spleen MDA levels on day 21. Manju et al. (36) have reported to observe similar effects in studies carried out in fish, *Anabas testudineus*. Their data demonstrate that TBARS content (lipid peroxidation product) in liver of fish fed the diets that were supplemented with % 0.5 and % 1 of curcumin decreased at the end of the second week.

The liver GSH-Px and GST, the kidney GSH-Px and the gill SOD activities of fish administered curcumin alone were determined to be increased in this study. This

Table 1. Levels of malondialdehyde (MDA) and activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and glutathione S-transferase (GST) in liver of the control and experimental groups. Significant differences ($p < 0.05$) between different treatment groups are indicated by different alphabetic superscripts.

Groups*	MDA (nmol/mg protein)	SOD (U/mg protein)	CAT (k/mg protein)	GSH-Px (U/mg protein)	GST (U/mg protein)
1	1.03 ± 0.10^{a}	2.66 ± 0.20^{a}	3.87 ± 0.36^{a}	2.48 ± 0.28^{b}	93.18 ± 13.50^{a}
2	0.95 ± 0.11^{a}	2.70 ± 0.17^{a}	3.92 ± 0.49^{a}	2.66 ± 0.37^{c}	109.54 ± 18.20^{b}
3	2.41 ± 0.16^{c}	5.17 ± 0.49^{c}	6.25 ± 0.82^{c}	1.14 ± 0.24^{a}	169.71 ± 22.10^{c}
4	1.16 ± 0.12^{b}	2.81 ± 0.43^{b}	4.54 ± 0.47^{b}	2.46 ± 0.45^{b}	112.23 ± 17.33^{b}
5	2.57 ± 0.17^{d}	5.43 ± 0.54^{c}	6.83 ± 0.58^{d}	1.08 ± 0.21^{a}	194.48 ± 26.47^{d}
6	1.19 ± 0.13^{b}	2.90 ± 0.36^{b}	4.61 ± 0.66^{b}	2.44 ± 0.26^{b}	114.19 ± 17.96^{b}

*1, control; 2, curcumin (100 mg/kg fish); 3, Malathion (0.5 mg/L); 4, Malathion (0.5 mg/L) plus curcumin (100 mg/kg fish/day); 5, Malathion (1 mg/L); 6, Malathion (1 mg/L) plus curcumin (100 mg/kg fish/day).
k: the first-order rate constant.

Table 2. Levels of malondialdehyde (MDA) and activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and glutathione S-transferase (GST) in kidney of the control and experimental groups. Significant differences ($p < 0.05$) between different treatment groups are indicated by different alphabetic superscripts.

Groups*	MDA (nmol/mg protein)	SOD (U/mg protein)	CAT (k/mg protein)	GSH-Px (U/mg protein)	GST (U/mg protein)
1	1.43 ± 0.14^{b}	2.41 ± 0.15^{a}	2.33 ± 0.15^{a}	1.41 ± 0.10^{d}	75.55 ± 8.33^{a}
2	1.16 ± 0.11^{a}	2.48 ± 0.13^{ab}	2.35 ± 0.17^{a}	1.50 ± 0.14^{e}	77.86 ± 11.20^{a}
3	2.76 ± 0.17^{d}	4.63 ± 0.27^{c}	3.89 ± 0.37^{b}	0.63 ± 0.06^{b}	137.55 ± 21.42^{c}
4	1.60 ± 0.12^{c}	2.50 ± 0.41^{ab}	2.38 ± 0.20^{a}	1.37 ± 0.12^{cd}	84.70 ± 13.97^{b}
5	2.97 ± 0.21^{e}	4.79 ± 0.38^{c}	4.34 ± 0.67^{c}	0.51 ± 0.08^{a}	139.63 ± 32.91^{c}
6	1.66 ± 0.15^{c}	2.56 ± 0.29^{b}	2.42 ± 0.46^{a}	1.33 ± 0.09^{c}	86.41 ± 19.23^{b}

*1, control; 2, curcumin (100 mg/kg fish); 3, Malathion (0.5 mg/L); 4, Malathion (0.5 mg/L) plus curcumin (100 mg/kg fish/day); 5, Malathion (1 mg/L); 6, Malathion (1 mg/L) plus curcumin (100 mg/kg fish/day).
k: the first-order rate constant.

Table 3. Levels of malondialdehyde (MDA) and activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and glutathione S-transferase (GST) in gill of the control and experimental groups. Significant differences ($p < 0.05$) between different treatment groups are indicated by different alphabetic superscripts.

Groups*	MDA (nmol/mg protein)	SOD (U/mg protein)	CAT (k/mg protein)	GSH-Px (U/mg protein)	GST (U/mg protein)
1	0.94 ± 0.10^{a}	1.52 ± 0.12^{a}	1.41 ± 0.16^{a}	1.10 ± 0.05^{b}	68.11 ± 12.53^{a}
2	0.93 ± 0.13^{a}	1.62 ± 0.10^{b}	1.46 ± 0.12^{a}	1.12 ± 0.08^{b}	70.79 ± 11.84^{a}
3	2.86 ± 0.23^{d}	3.84 ± 0.49^{e}	2.79 ± 0.28^{c}	0.69 ± 0.09^{a}	154.90 ± 21.81^{d}
4	1.17 ± 0.11^{b}	1.91 ± 0.24^{d}	1.60 ± 0.31^{b}	1.08 ± 0.11^{b}	79.60 ± 13.74^{b}
5	3.02 ± 0.21^{e}	3.99 ± 0.61^{e}	2.92 ± 0.37^{d}	0.67 ± 0.10^{a}	165.34 ± 28.06^{e}
6	1.30 ± 0.18^{c}	1.73 ± 0.17^{c}	1.64 ± 0.35^{b}	1.09 ± 0.08^{b}	92.51 ± 13.45^{c}

*1, control; 2, curcumin (100 mg/kg fish); 3, Malathion (0.5 mg/L); 4, Malathion (0.5 mg/L) plus curcumin (100 mg/kg fish/day); 5, Malathion (1 mg/L); 6, Malathion (1 mg/L) plus curcumin (100 mg/kg fish/day).
k: the first-order rate constant.

increase was also observed by Mişe Yonar et al. (35) in a study carried out in rainbow trout. They documented significant increases of GSH-Px, GR, and GST activity and GSH level in liver, kidney, and spleen. Similar results were obtained by Manju et al. (36), who evaluated antioxidant responses in *Anabas testudineus* after administered curcumin. In their study, SOD and CAT activity in liver increased in fish fed the diets that were supplemented with % 0.5 and % 1 of curcumin. Consistent with previous investigations (35,36), the increases determined in this study may be explained with possibly enhancement of antioxidant capacity in the tissues.

The plasma MDA levels of the groups that administered malathion alone were determined to be increased in this study. When compared with the control group, this increase was found to be statistically significant. On the other hand, the SOD, CAT, and GST activities in the tissues increased, whereas GSH-Px activity decreased. The changes in the indicated parameters showed that the antioxidant enzymes activities were insufficient in the compensation of free radicals generated at a high level upon the administration of malathion at different concentrations for the indicated period. For, the increase in the MDA level also confirms this situation. The decrease or increase in the activities of the enzymes can be explained either with their consumption and induction during the conversion of free radicals into less harmful or harmless metabolites or secondarily with the direct inhibitory or stimulatory effect of ROS that may be caused by malathion. In a study carried out by Mişe Yonar et al. (4), malathion was reported to cause increase in the SOD and CAT activities and the MDA and GSH levels in carp. Similar results were reported by Mişe Yonar (18), who evaluated the effects of malathion on *Cyprinus carpio*. Huculeci et al. (17) observed increased antioxidant enzyme activity of GST in kidney and intestine of the freshwater goldfish after exposure to malathion in concentrations of 0.05 mg/L. Ural et al. (37) documented an increase of the tissue GST activity in carp after malathion exposure. This status can be most likely explained with an excessive production of ROS, which could be related to antioxidant enzyme leakage or exceeding of antioxidant capacity by the amount of free radicals. This was confirmed by the increase in the MDA level and the decrease in the GSH-Px activity in the tissues.

The present investigation shows that curcumin possesses an antioxidant activity, which may be attributed to its protective action on lipid peroxidation and to the enhancing effect on antioxidant defence that might further contribute to the protection against oxidative stress in malathion-induced toxicity. The primary mechanism of this effect of curcumin may involve direct scavenging of O_2^-, H_2O_2 and nitric oxide radicals (24,36). The other mechanism by which curcumin protects oxidative stress in endothelial cells is by induction of heme oxygenase-1 (36).

In conclusion, malathion caused oxidative stress in carp. The administration of curcumin with known antioxidant property caused alterations in oxidative stress parameters and alleviated severity of oxidative stress. On the other hand, the administration curcumin alone enhanced antioxidant capacity in the tissues. Therefore, curcumin may be used as an antioxidant in fish.

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