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Effects of Deltamethrin on Antioxidant Status and Oxidative Stress Biomarkers in Freshwater Mussel, *Unio elongatulus eucirrus*

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Abstract In this study, the effects of deltamethrin on antioxidant status and oxidative stress biomarkers in digestive gland and gill of freshwater mussel, *Unio elongatulus eucirrus*, was examined. Deltamethrin was applied at concentrations of 25, 50, 100, 200, 400, 800 and 1,600 μg L^{-1}, for 1, 24, 48, 72 and 96 h. With increasing deltamethrin concentrations, the mussels exposed duration 1–96 h significantly increased lipid peroxidation, which might be associated to decreased levels of reduced glutathione and catalase activity in digestive gland and gill of freshwater mussel ($p < 0.05$ for each cases). Negative correlations were observed between the lipid peroxidation and glutathione or catalase levels after deltamethrin exposure, indicating a protective role of glutathione and catalase against lipid peroxidation, suggesting the use of these antioxidants as a potential biomarker of toxicity associated with contaminant exposure in freshwater mussels.

Keywords Freshwater mussel · *Unio elongatulus eucirrus* · Deltamethrin · Oxidative stress · Antioxidants · Biomarkers

The increasing use of synthetic pesticides is improving worldwide pollution risks. The synthetic pyrethroids are among the most potent and effective insecticides available (Casida et al. 1983), accounting for more than 30% of the world market in insecticides. They are less persistent and less toxic to mammals and birds. One of the pyrethroids that have found wide acceptability is deltamethrin. It is extensively used in agriculture and forestry because of its high activity against a broad spectrum of insect pests (Glickman and Lech 1982).

Pesticides applied to the land may be washed into surface waters and may kill or at least adversely influence the life of aquatic organisms (Köprücü et al. 2006). Synthetic pyrethroids have been found to be highly toxic to fish (Datta and Kaviraj 2003; Köprücü and Aydın 2004), zooplankton communities and some beneficial aquatic arthropods (Tidou et al. 1992). Effects of deltamethrin on nervous, haematological and respiratory systems in fishes are reported (Golow and Godzi 1994). Deltamethrin have proved to alter the filtration and pumping activity of mussels (Kontreczky et al. 1997). The environmental fate and effects of synthetic pyrethroid insecticides have been summarized by Hill (1989).

Study of deltamethrin-induced oxidative stress and its influence on various antioxidants of fish and other aquatic organisms could provide useful information on the ecotoxicological consequences of deltamethrin use. Several studies have shown that the antioxidants of fish and mussels could be used as biomarkers of exposure to aquatic pollutants. A review of the literature reveals that there is a paucity of information on deltamethrin-induced oxidative stress and its effects on various enzymatic antioxidants in freshwater mussels (Charissou et al. 2004; Almeida et al. 2005; Parvez and Raisuddin 2006).

In recent years, there has been considerable interest in the use of biochemical indices within bivalve molluscs. Mussels have a number of properties which make them useful sentinels for chemical pollution. By filtering large quantities water, mussels may be exposed to large quantities of pollutants even when their concentrations are quite small (Robillard et al. 2003).

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In Turkey, deltamethrin is a commonly used pesticide for pest control in the agricultural fields around freshwater reservoirs. Therefore, the present study has aimed to investigate effects of deltamethrin on antioxidant status and oxidative stress biomarkers in digestive gland and gill of freshwater mussel, *Unio elongatulus eucirrus*. An attempt has also been made to access usefulness of these parameters as biomarkers of exposure to deltamethrin.

Materials and Methods

In the present study, a semi-static acute toxicity bioassay was performed according to the standard method (APHA 1985). Deltamethrin was applied at sub-acute doses of 25, 50, 100, 200, 400, 800 and 1,600 μg L^{-1}, for 1, 24, 48, 72 and 96 h.

Deltamethrin, (S)-alpha-cyano-3-phenoxybenzyl (1R, 3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropancarboxylate, was obtained from Roussel Uclaf, in form of DECIS 2.5 EC (purity 2.5%, dissolved in 97.5% acetone).

Adult specimens of freshwater mussels, weighing 24–26 g and total length 5.8–6.1 cm, were obtained from the Pertek Region of Keban Dam Lake in Turkey. They were brought to the laboratory and acclimatized to laboratory conditions for 7 days. Water temperature in the aquaria was maintained at 12 ± 1°C using a heater and the mussels were subjected to a 12 h photoperiod using fluorescent lights. Mussels were fed with trout feed during adaptation, but they were not fed during the last 24 h of adaptation and throughout the duration of the test. Before starting the test, all experimental aquaria (280 L) were cleaned and filled with 270 L of dechlorinated tap water. Ten mussels were transferred into each aquarium. Five replicates were used in the control and test series. The experimental water was kept in the tank for 24 h before deltamethrin was added.

Water quality characteristics in the control units were determined according to APHA (1985). Dissolved oxygen and pH were determined by a digital oxygen meter and a pH meter. The mean quality parameters of water used for preparation of test solutions were as follows; dissolved oxygen 8.3 ± 0.4 mg L^{-1}, pH 7.1 ± 0.2, alkalinity 138 ± 12 mg L^{-1} and total hardness 187 ± 15 mg L^{-1} as $CaCO_3$.

Seven different concentrations of deltamethrin and a control with five replicates were used in the test series. Two control groups received acetone at a concentration used in the dilution of the maximum deltamethrin concentration. Exceeding aeration was applied to the aquarium for 2 h in order to obtain a homogeneous concentration of the toxic compound, and then ten mussels were transferred into each aquarium. Mortality was assessed at 1, 24, 48, 72 and 96 h after the start and dead mussels were removed immediately.

After tissue samples rinsing with cold 0.09% NaCl solution and filtering to remove fluid, the tissue samples were weighed meticulously. The homogenization of tissues was carried out in a Sorvall homogenizer with a buffer containing 1.15% KCl to obtain 1:10 (w/v) whole homogenate. The homogenates were centrifuged at 18,000*g* (+4°C) for 30 min to determine malondialdehyde, reduced glutathione concentrations and catalase activities. The concentrations of malondialdehyde as a marker of lipid peroxidation were determined according to the method of Ohkawa et al. (1979) based on the reaction with thiobarbituric acid, and were expressed as nmol/g tissue. Catalase activity was determined according to the method of Aebi (1974). Tissue glutathione concentration was measured by an assay using the dithionitrobenzoic acid recycling method described by Ellman (1959). Protein concentrations were measured according to Lowry et al. (1951).

Statistical analyses were performed with the SPSS 10.1 computer program (SPSS Inc. Chicago, IL, USA). The results were expressed as mean ± SE. Significant differences between groups were analyzed by one-way analysis of variance and Duncan's post hoc test where appropriate. The significance of the results was ascertained at $p < 0.05$.

Results and Discussion

The results show that with increasing deltamethrin concentrations, the mussels exposed duration 1–96 h significantly increased lipid peroxidation (Table 1), which might be associated to decreased levels of reduced glutathione (Table 2) and catalase (Table 3) activity in digestive gland and gill of freshwater mussel ($p < 0.05$ for each cases). Negative correlations were observed between the lipid peroxidation and glutathione or catalase levels after deltamethrin exposure, indicating a protective role of glutathione and catalase against lipid peroxidation, and suggesting the use of these antioxidants as a potential biomarker of toxicity associated with contaminant exposure in freshwater mussels.

Deltamethrin is known to after cell metabolism in various ways, with a potential genotoxic risk, including DNA damage and micronucleus induction in human lymphocytes (Scassellati et al. 1994). The present findings implicate a role of oxidative stress and free radical formation in these effects. Studies in rodents have demonstrated that absorbed deltamethrin is readily metabolized and excreted, elimination is achieved within 2–4 days. The tissue residue levels are reported to be generally very low, except in fat, where slightly higher residues occurred. The major metabolic reactions ascribed to deltamethrin metabolism are oxidations mediated by the microsomal monooxygenase system. The degredation pathways in cows, poultry, and

Table 1 Lipid peroxidation levels in digestive glands and gills of freshwater mussel, *Unio elongatulus eucirrus*, exposed to sub-lethal doses of deltamethrin, for 1–96 h

Concentrations (μg L^{-1})	Lipid peroxidation levels (nmol/g tissue)									
	In digestive glands (h)					In gills (h)				
	1	24	48	72	96	1	24	48	72	96
Control	1.50 ± 0.01^{a}	1.51 ± 0.01^{a}	1.53 ± 0.02^{a}	1.52 ± 0.02^{a}	1.54 ± 0.03^{a}	0.48 ± 0.01^{a}	0.49 ± 0.01^{a}	0.51 ± 0.02^{a}	0.53 ± 0.03^{a}	0.54 ± 0.03^{a}
25	1.56 ± 0.03^{a}	1.59 ± 0.03^{a}	1.64 ± 0.05^{a}	1.61 ± 0.04^{a}	1.58 ± 0.03^{a}	0.56 ± 0.03^{a}	0.59 ± 0.04^{a}	0.63 ± 0.05^{a}	0.62 ± 0.04^{a}	0.58 ± 0.04^{a}
50	1.62 ± 0.04^{a}	1.66 ± 0.05^{a}	1.72 ± 0.07^{a}	1.69 ± 0.05^{a}	1.65 ± 0.04^{a}	0.63 ± 0.04^{a}	0.67 ± 0.05^{a}	0.72 ± 0.06^{a}	0.70 ± 0.06^{a}	0.66 ± 0.05^{a}
100	1.69 ± 0.05^{a}	1.74 ± 0.07^{a}	1.82 ± 0.08^{a}	1.78 ± 0.08^{a}	1.73 ± 0.07^{a}	0.70 ± 0.06^{b}	0.76 ± 0.07^{ab}	0.84 ± 0.08^{a}	0.81 ± 0.08^{a}	0.74 ± 0.07^{ab}
200	1.77 ± 0.07^{b}	1.93 ± 0.08^{ab}	2.07 ± 0.10^{a}	1.97 ± 0.09^{ab}	1.82 ± 0.08^{ab}	0.79 ± 0.07^{c}	0.89 ± 0.08^{bc}	1.05 ± 0.10^{a}	0.92 ± 0.09^{ab}	0.83 ± 0.08^{bc}
400	1.86 ± 0.08^{b}	2.08 ± 0.11^{ab}	2.24 ± 0.14^{a}	2.15 ± 0.13^{ab}	1.93 ± 0.09^{ab}	0.88 ± 0.08^{b}	1.09 ± 0.10^{a}	1.22 ± 0.12^{a}	1.13 ± 0.11^{a}	0.93 ± 0.09^{b}
800	1.99 ± 0.12^{c}	2.29 ± 0.15^{abc}	2.43 ± 0.17^{a}	2.36 ± 0.16^{ab}	2.08 ± 0.14^{c}	1.04 ± 0.11^{c}	1.28 ± 0.13^{ab}	1.41 ± 0.15^{a}	1.32 ± 0.14^{ab}	1.15 ± 0.13^{bc}
1,600	2.18 ± 0.16^{b}	2.39 ± 0.18^{ab}	2.61 ± 0.20^{a}	2.45 ± 0.19^{ab}	2.25 ± 0.17^{ab}	1.22 ± 0.14^{c}	1.43 ± 0.15^{ab}	1.58 ± 0.16^{a}	1.49 ± 0.15^{ab}	1.29 ± 0.14^{bc}
p-value	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$

Values are expressed as mean ± SE (n = 10); Lipid peroxidation levels in rows with different letters significantly differ ($p < 0.05$ for each cases)

Table 2 Glutathione levels in digestive glands and gills of freshwater mussel, *Unio elongatulus eucirrus*, exposed to sub-lethal doses of deltamethrin, for 1–96 h

Concentrations (μg L^{-1})	Glutathione levels (nmol/g tissue)									
	In digestive glands (h)					In gills (h)				
	1	24	48	72	96	1	24	48	72	96
Control	$1{,}094 \pm 16.5^{a}$	$1{,}079 \pm 14.0^{a}$	$1{,}086 \pm 15.9^{a}$	$1{,}090 \pm 16.3^{a}$	$1{,}086 \pm 15.6^{a}$	868 ± 21.3^{a}	837 ± 19.3^{a}	848 ± 20.4^{a}	861 ± 20.5^{a}	850 ± 19.0^{a}
25	$1{,}077 \pm 33.5^{a}$	981 ± 26.7^{ab}	891 ± 19.9^{b}	976 ± 25.0^{ab}	1070 ± 32.8^{a}	840 ± 12.4^{a}	771 ± 16.2^{a}	727 ± 14.5^{a}	764 ± 16.0^{a}	835 ± 11.9^{a}
50	$1{,}043 \pm 25.8^{a}$	930 ± 19.8^{ab}	838 ± 12.1^{b}	922 ± 18.0^{ab}	1036 ± 24.5^{a}	800 ± 11.2^{a}	722 ± 14.8^{ab}	679 ± 11.7^{b}	717 ± 14.3^{ab}	792 ± 11.0^{ab}
100	942 ± 19.1^{a}	848 ± 10.5^{ab}	763 ± 6.46^{b}	835 ± 9.65^{ab}	926 ± 18.2^{a}	742 ± 9.97^{a}	659 ± 11.5^{ab}	610 ± 9.72^{b}	650 ± 11.1^{ab}	732 ± 9.64^{a}
200	858 ± 15.3^{a}	773 ± 7.98^{ab}	700 ± 5.42^{b}	757 ± 7.51^{ab}	839 ± 12.2^{a}	666 ± 7.65^{a}	581 ± 7.65^{ab}	534 ± 6.49^{b}	571 ± 6.85^{ab}	653 ± 7.26^{a}
400	723 ± 10.7^{a}	651 ± 4.46^{ab}	585 ± 2.34^{b}	631 ± 3.99^{ab}	702 ± 8.97^{a}	580 ± 5.72^{a}	488 ± 5.72^{bc}	440 ± 4.97^{c}	474 ± 5.41^{c}	566 ± 5.43^{ab}
800	655 ± 6.94^{a}	590 ± 3.01^{ab}	531 ± 1.34^{b}	566 ± 2.96^{ab}	631 ± 6.26^{a}	485 ± 4.67^{a}	399 ± 3.97^{b}	356 ± 3.87^{b}	382 ± 3.75^{b}	469 ± 4.55^{a}
1,600	454 ± 3.95^{a}	408 ± 1.43^{ab}	368 ± 1.12^{b}	383 ± 1.31^{b}	426 ± 3.73^{ab}	360 ± 3.41^{a}	343 ± 2.98^{ab}	300 ± 1.22^{b}	330 ± 2.65^{ab}	347 ± 4.21^{ab}
p-value	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$

Values are expressed as mean ± standard error (n = 10); Glutathione levels in rows with different letters significantly differ ($p < 0.05$ for each cases)

Table 3 Catalase activity in digestive glands and gills of freshwater mussel, *Unio elongatulus eucirrus*, exposed to sub-lethal doses of deltamethrin, for 1–96 h

Concentrations (μg L^{-1})	Catalase activity (nmol/g tissue)									
	In digestive glands (h)					In gills (h)				
	1	24	48	72	96	1	24	48	72	96
Control	161 ± 1.95^{a}	163 ± 2.01^{a}	164 ± 2.50^{a}	167 ± 2.65^{a}	165 ± 2.59^{a}	112 ± 1.97^{a}	114 ± 2.12^{a}	116 ± 1.58^{a}	115 ± 1.40^{a}	114 ± 2.20^{a}
25	126 ± 4.89^{a}	120 ± 3.76^{a}	110 ± 3.35^{a}	119 ± 3.58^{a}	123 ± 4.67^{a}	87 ± 2.05^{a}	80 ± 1.80^{ab}	71 ± 1.46^{b}	79 ± 1.84^{ab}	85 ± 1.90^{a}
50	123 ± 4.50^{a}	115 ± 4.35^{a}	106 ± 3.17^{a}	114 ± 4.16^{a}	119 ± 4.40^{a}	86 ± 1.96^{a}	77 ± 1.65^{ab}	68 ± 1.32^{b}	76 ± 1.70^{ab}	83 ± 1.85^{a}
100	119 ± 4.05^{a}	110 ± 3.56^{ab}	99 ± 2.83^{b}	108 ± 3.15^{ab}	114 ± 3.99^{ab}	82 ± 1.85^{a}	72 ± 1.50^{ab}	63 ± 1.30^{b}	71 ± 1.46^{ab}	79 ± 1.80^{a}
200	115 ± 2.84^{a}	104 ± 2.70^{ab}	92 ± 2.67^{b}	103 ± 2.65^{ab}	109 ± 2.78^{a}	78 ± 1.79^{a}	66 ± 1.48^{bc}	57 ± 1.23^{c}	65 ± 1.42^{bc}	74 ± 1.66^{ab}
400	110 ± 2.45^{a}	97 ± 2.13^{a}	82 ± 2.06^{b}	97 ± 2.04^{a}	105 ± 2.30^{a}	73 ± 1.64^{a}	59 ± 1.30^{bc}	49 ± 1.19^{d}	59 ± 1.27^{c}	69 ± 1.44^{ab}
800	102 ± 2.12^{a}	89 ± 2.00^{a}	71 ± 1.91^{b}	88 ± 1.96^{a}	97 ± 2.05^{a}	66 ± 1.38^{a}	53 ± 1.20^{bc}	40 ± 1.00^{d}	50 ± 1.24^{c}	61 ± 1.29^{ab}
1,600	95 ± 1.86^{a}	76 ± 1.70^{bc}	58 ± 1.47^{d}	75 ± 1.62^{c}	89 ± 1.80^{ab}	58 ± 1.12^{a}	44 ± 1.11^{b}	27 ± 0.85^{d}	40 ± 1.06^{b}	52 ± 1.18^{a}
p-value	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$

Values are expressed as mean ± standard error (n = 10); Catalase activity in rows with different letters significantly differ ($p < 0.05$ for each cases)

fish are almost similar to those in rodents (IPCS 1989). However, comparative in vivo and in vitro metabolic studies have shown that fish have a lower capacity to metabolize and eliminate pyrethroid insecticides (Glickman and Lech 1982). This is reflected in the present investigation, where deltamethrin induced peroxidative damage in digestive gland and gill in particular during a short-term sub-acute exposure regimen.

The extent of lipid peroxidation is determined by the balance between the production of oxidants and the removal and scavenging of those oxidants by antioxidants (Filho 1996). Lipid peroxidation has been extensively used as a marker of oxidative stress (Huggett et al. 1992). In the present investigation, we also showed that lipid peroxidation estimation could provide useful information about the exposure to aquatic pollutants. However, the response may be nonspesific in terms of type of pollutants.

Use of glutathione and catalase as biomarkers of exposure to organic xenobiotics has gained credence in aquatic pollution biomonitoring (Almeida et al. 2005). The activity of glutathione and catalase was found to be significantly decreased in digestive gland and gill. This decrease in catalase activity could be due to flux of superoxide radicals, which have been reported to inhibit catalase activity (Pandey et al. 2001). Reduced glutathione is the man nonprotein thiol and one of the main reductants found in cell (Siegers 1989). It possesses antioxidant properties and its protective role against oxidative stress induced toxicity in aquatic animals is well established (Hasspielar et al. 1994).

Our results show decrease in glutathione and catalase levels in digestive gland and gill of freshwater mussel. This demonstrates a protective response in freshwater mussel toward exposure to an oxidative stress inducing deltamethrin. However, the induction pattern of glutathione and catalase under long-term exposure conditions needs to be investigated.

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