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SUCCINATE DEHYDROGENASE ACTIVITY IN HEART AND KIDNEY TISSUES AS A BIOMARKER OF DELTAMETHRIN TOXICITY IN ANABAS TESTUDINEUS

Environmental Science

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ABSTRACT

Anabas testudineus, a freshwater fish was exposed for 21 days to a commercial formulation of type II (α-cyano) synthetic pyrethroid deltamethrin at sublethal concentrations of 0.0078 and 0.00078 mg L-1, that represented 10% and 1%, respectively, of its 96 h LC50 concentration for this fish. Succinate dehydrogenase (SDH) enzyme activity in heart and kidney tissues of control and pesticide-exposed fish were estimated following standard protocols. When compared with those of control fish, significant depletion of SDH activity was observed in heart and kidney tissues at both lower and higher sublethal concentrations. It is concluded that decreased SDH activity in heart and kidney, could serve as a fairly specific biomarker for deltamethrin. Further, deltamethrin could cause significant reductions in SDH activity at fairly low and environmentally relevant concentrations, thereby identifying it as a chemical of environmental concern.

KEYWORDS

Fish; Type-II pyrethroid; Succinate dehydrogenase

SUMMARY

A study was carried out to investigate the sublethal effects of synthetic pyrethroids deltamethrin on succinate dehydrogenase (SDH) enzyme activity in heart and kidney tissues of *Anabas testudineus* after an exposure period of 21 days. SDH level was reduced significantly in heart and kidney tissues at higher (10% 96 h LC_{50}) as well as lower (1% 96 h LC_{50}) deltamethrin concentrations. These significant decreasing effects on SDH activity in both the tissues of *Anabas testudineus* indicated that deltamethrin have severe impact on the respiratory enzyme disrupting the normal TCA cycle resulting in disturbances in the normal respiratory mechanism of the fish. Suppression of SDH activity also reflected the inhibition in the conversion of succinate to fumarate and also a metabolic shift from aerobiosis to anaerobiosis. Results obtained in the present study also suggest that inhibition of SDH activity in heart and kidney tissues emerged as a biomarker with potentially higher specificity for deltamethrin in *Anabas testudineus*.

1. INTRODUCTION

Pesticides can enter aquatic ecosystems via agricultural runoff and produce toxic effects in fish and other aquatic organisms (Das and Mukherjee, 2003). Among the major classes of pesticides used to control pests, synthetic pyrethroids have largely replaced organochlorines, organophosphates and carbamates because of their rapid biodegradability and non-persistent nature (Aydin *et al.,* 2005). While synthetic pyrethroids are less toxic to birds and mammals, they are reported to be highly neurotoxic to fish (Brander *et al.,* 2016). Extensive use of these pesticides, therefore, poses a severe threat to fish and other aquatic taxa (Ahmad *et al.,* 2012). Moreover, excess use of pyrethroids has also been reported to cause impairment of many biochemical functions in fish (Begum, 2007). Among the pyrethroids, the α-cyano-substituted type II synthetic pyrethroids are more neurotoxic than the type I non cyano-substituted pyrethroids (Kaviraj and Gupta, 2014). The type II synthetic pyrethroid deltamethrin is commonly used in the tea plantations of Assam, as well as in those of Dooars and Darjeeling in West Bengal, India (Gurusubramanian *et al.,* 2008). Thus, it may be of interest to assess the effects of this pyrethroid on native fish species of ecological and economic importance.

Sub lethal effects of toxic substances are biochemical in origin as most of the toxicants exert their effects by reacting with enzymes or other functional components of the cell (Reddy *et al.,* 2011). Therefore, changes in the activities of enzymes have been used as possible tools for aquatic toxicological research. Teleost fish may be a good indicator of contamination of aquatic systems as their biochemical responses are quite similar to those of mammals (Banaee *et al.,* 2008).

A wide range of enzymatic and non-enzymatic biomarkers in response to synthetic pyrethroid exposure have been investigated in different fish tissues that commonly include liver, muscle, gill, and less frequently kidney (Sayeed *et al.,* 2003; Mushigeri and David, 2004; Singh and Singh, 2004; Begum, 2007; Vani *et al.,* 2011, 2012; Loteste *et al.,* 2013; Sapana Devi and Gupta, 2014). On the contrary, information on the effects of pesticide stress on enzyme activity is relatively less in brain, and very rare in cardiac tissue. In view of the above, effects of a type II synthetic pyrethroid on heart and kidney tissues of *A. testudineus* were included in the present study. The investigated enzyme, succinate dehydrogenase (SDH), an essential component of mitochondrial respiratory chain (Brenet *et al.,* 2021) - also known as Electron Transport Chain Complex II - is an enzyme that catalyzes succinate to fumarate in the tricarboxylic acid (TCA) cycle. Interest in this complex has been renewed in recent years due to its role in several human diseases including cancers and neurodegeneration (Rutter *et al.,* 2010; Moosavi *et al.,* 2019). The test fish, *Anabas testudineus*, Bloch 1792, is an important food fish in North, East and Northeast India as well as in other countries of Southeast Asia.

In the context of the above, this study investigates the deleterious effects of sublethal concentrations of deltamethrin 2.8% E.C. (trade name-Decis) on the activities of succinate dehydrogenase (SDH) enzyme in heart and kidney tissues of *A. testudineus*.

2. MATERIALS AND METHODS

2.1. Ethical procedures

All fish handling and maintenance was conducted under the approval of the University animal ethics committee.

2.2. Procurement of fish and pesticides

Specimens of *Anabas testudineus* weighing 12±2 g and length 8-10 cm were collected from unpolluted ponds in Cachar district, Assam, which are located far away from agricultural lands where pesticides are applied. These were then brought to the laboratory, and immediately transferred to glass aquaria after dipping them in 0.1% potassium permanganate solution to cure any existing infection. They were fed commercial fish pellets (Tokyu-Spirulina, special fish food, floating type containing 32% crude protein, 4% crude fat, 5% crude fibre, 10% crude ash, 9% moisture and 31% nitrogen free extract) and acclimatized for 15 days prior to the commencement of the experiment. Water was aerated and renewed every day. Commercial grade deltamethrin 2.8% E.C. (Trade name Decis - Agrevo India Ltd.) was purchased from a local agrochemical seller.

2.3. Analysis of deltamethrin concentration in 2.8% E.C.

Standard deltamethrin pesticide of high purity (99.6%) was purchased from Sigma-Aldrich Laborchemikalien GmbH D-30918 Seelze, Quality Management SA-LC. HPLC grade water, acetone and acetonitrile were purchased from a supplier in Imphal, Manipur, India. All the solvents were glass redistilled by fractional distillation apparatus (Gellenkamp, UK) before use. Nominal concentrations of 1ppm, 0.5ppm and 0.1 ppm of deltamethrin were made following dilution from the stock solution of 1000 ppm. The solutions were centrifuged (Remi Instrument Ltd.) and the supernatant were then used for HPLC analysis. The standards and samples of different

concentrations of 1ppm, 0.5ppm and 0.1 ppm respectively were analyzed by using High performance liquid chromatography (HPLC-Agilent 1260 Infinity) having diode-array detector (DAD). Identification of the pesticide was carried out by comparing sample retention times with those obtained for standards from Sigma-Aldrich Laborchemikalien GmbH D-30918 Seelze. The areas of sample peaks were compared with those of the standards to quantify the concentrations of deltamethrin. The wavelength of 225nm was observed to be the optimum wavelength for identification of deltamethrin. The HPLC conditions being used to analyze deltamethrin is presented in Table 1.

2.4. Enzyme assay

In order to evaluate the sublethal effects of deltamethrin on the activities of succinate dehydrogenase (SDH) enzyme in heart and kidney tissues, specimens of *Anabas testudineus* were exposed to 10% and 1% of the 96 h LC_{50} of deltamethrin, the nominal concentrations of which were 0.007 and 0.0007 mg L^{-1}, with measured concentrations of 0.0078 and 0.00078 mg L^{-1}, respectively. A control was maintained in aerated unchlorinated tap water without added pesticide. Nine replicates were used in each treatment and the control. The fishes were fed with 'Tokyu' fish food throughout the test period. Control and exposed fishes were sacrificed after a 21 day exposure period (Fırat *et al.,* 2011; Ahmad *et al.,* 2012) and heart and kidney tissues were dissected out for enzyme assays. Estimation of SDH was made spectrophotometrically and expressed as mg formazan formed/minute/mg fresh tissue weight after Kun and Abood (1949) as modified in Beatty *et al.* (1966). The regression formula was: x= 1.866 + 522.21y.

2.5. Physico-chemical properties of water

Temperature, pH and electrical conductivity of the test medium were measured with a mercury bulb thermometer, a digital pH meter, and a conductivity-TDS meter, respectively. Dissolved oxygen was estimated iodometrically by Winkler's method.

2.6. Data analysis

Enzyme assay data were first tested for normality (Kolmogorov-Smirnov test) before applying One-Way Analysis of Variance (ANOVA) to determine significant differences among control and experimental groups. If a difference was detected at $p<0.05$, Tukey test was applied for multiple comparisons.

3. RESULTS AND DISCUSSION

3.1. Physico-chemical properties of water

The physico-chemical properties of the test water were: temperature: 22.1 – 22.3 ºC; pH 6.54 – 6.73; electrical conductivity: 80.9 – 82.6 µS cm^{-1}; and dissolved oxygen: 6.72 – 7.09 mg L^{-1}.

Table 1: The HPLC conditions being used to analyze different concentrations of deltamethrin.

HPLC conditions	Deltamethrin
Mobile phase ratio	Acetonitrile : Water (90:10)
Flow rate	1 ml/min
Wavelength	225nm
Injection (µL)	20 µL
Column	Zorbax Eclipse Plus C18 (4.6m X 100mm 3.5 – micron)
Run Time	15 min

3.2. Enzyme assays

Comparisons of SDH enzyme activity in heart and kidney tissues of deltamethrin- treated *Anabas testudineus* by one-way ANOVA revealed statistically significant depletion of the enzyme in heart and kidney tissues of fish treated with the pesticide (heart: $p<0.001$ and kidney: $p<0.001$). Multiple comparisons with Tukey tests revealed that when compared with the control, inhibition of SDH activity by deltamethrin at 0.0078 mg L^{-1} and 0.00078 mg L^{-1} were significant in both the tissues i.e., heart and kidney.

Table 2. Reductions in SDH (mg formazan formed/min/mg fresh tissue) in *Anabas testudineus* tissues (heart and kidney) exposed for 21 days to sublethal concentrations of deltamethrin.

Concentration (mg l-1)	SDH (heart)	% reduction from control	SDH (kidney)	% reduction from control
Control	0.063±0.002 a	NA	0.08±0.01a	NA
Deltamethrin-10% of 96 h LC50 (0.0078)	0.037±0.004 b	41.27	0.047±0.01 b	41.25
Deltamethrin-10% of 96 h LC50 (0.0078)	0.047±0.01 b	25.39	0.06±0.003 b	25

NA: Not applicable

Values are expressed as mean ± S.E.; Values with different superscript letters in a column are significantly different from one another at $p \leq 0.05$.

The reductions in SDH activity from control in deltamethrin challenged fish ranged from 25.39-41.27% in heart and 25-41.25% in kidney tissues (Table 2), revealing that deltamethrin at relatively low concentrations (0.00078 mg L^{-1})had severe similar significant effect on heart and kidney, with percent reduction (25 – 41.2 %), both the tissues are affected. Exposure to acute (1ppm) and sublethal (0.1ppm) concentrations of deltamethrin reduced SDH activity in liver and muscle tissues of *Labeo rohita* by 34.5-62.7% (Rathnamma et al. 2009). In the present study deltamethrin was found to significantly decrease SDH activity in heart and kidney tissues at an extremely low concentration of 0.00078 mg L^{-1}, which is environmentally relevant in view of the presence of deltamethrin residues in rain water (0.1-0.8 ppb), groundwater (0.017-0.061 ppm), soil (0.0016-0.019 ppm), and vegetables (0.024-0.173 ppm) (Vig et al. 2001; Kumari et al. 2002, 2007, 2008) in different parts of India. Depletion of SDH level in kidney tissue of *Labeo rohita* exposed to cypermethrin indicated switch-over to anaerobic metabolism which in turn affected the normal physiological functioning of different tissues thereby attributing a repressor effect in their synthesis (Das and Mukherjee 2003).

Inhibition of SDH activity in heart and kidney tissues of *Anabas testudineus* in the present study, at extremely low deltamethrin concentrations suggests reduced conversion of succinate to fumarate, which reflects impairment of TCA cycle in these tissues. Similar decrease in SDH activity in various tissues of *Cyprinus carpio* treated with lethal concentrations of group-II pyrethroids (deltamethrin, cypermethrin, fenvalerate and fluvalinate) for a period of 72 h indicated inhibition of SDH at mitochondrial level (Kamalaveni et al. 2001). Inhibition of SDH activity in heart tissue of *Anabas testudineus* in the present study suggests that like other tissues, the normal functioning of the respiratory enzyme SDH in heart may be altered due to deltamethrin exposure thereby disrupting the normal tricarboxylic acid cycle (TCA) by inhibiting the conversion of succinate to fumarate (Singh and Singh 2004). It is possible that this can have implications in the functioning of the cardiac muscles.

Similarly, impaired energy metabolism in kidney could mean less effective glomerular filtration. It was also seen that SDH activity in heart and kidney were affected at low ppb (0.78 µg L^{-1}) levels of deltamethrin, which is a fairly low and environmentally relevant concentrations, thereby identifying it as a chemical of environmental concern.

4. CONCLUSIONS

While a large number of biomarkers at different levels have been identified to characterize pesticide, and more specifically, synthetic pyrethroid toxicity, efforts are on to find biomarkers with increased specificity (Kaviraj and Gupta 2014). Results obtained in the present study suggest that inhibition of SDH activity in heart and kidney tissues emerged as a biomarker with potentially higher specificity for deltamethrin in *Anabas testudineus*.

DECLARATION:

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Conflict of Interest: The authors declared that there is no conflict of interest in this research work.

Ethics Approval: Not Applicable, because this research work is related to fish tissue only.

Consent to Participate: Not Applicable

Consent for publication: Not Applicable

Availability of data and material: All data and materials obtained during this research work are submitted along with this manuscript.

Code availability: Not Applicable

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