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Short term effect of thiamethoxam on glutamate dehydrogenase (GDH) activity in Snake head, *Ophiocephalus punctatus*

Authors:
Arun M. Chilke

Institution:
Division of Toxicology and Biomonitoring, Department of Zoology,
Shree Shivaji Arts, Commerce and Science College, Rajura-442905 (India).

Corresponding author:
Arun M. Chilke

Email:
achilke.2011@rediffmail.com

Phone No:
917173 222215

ABSTRACT:

An organochloride, thiamothoxam is used commercially for controlling the agricultural pest. In the present study it was observed that at sub-lethal concentration (29.483 ppm) the GDH activity in the liver, kidney, gill and muscle of *Ophiocephalus punctatus* increased non-significantly from 24hrs to 96hrs of exposure. The maximum activity in all these organs was at 96 hrs. It has been concluded that thiamethoxam at sub-lethal concentration may alter the glutamate metabolism in all the tissues by enhancing the GDH activity in short duration of exposure.

Keywords:
Thiamethoxam, GDH, Snake heads, Ophiocephalus.

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INTRODUCION

Pesticide usage all over the world has increased dramatically during the past few decades, coinciding with changes in farming and intensive agriculture practices. Hence the environmental pollution caused by pesticides, especially in aquatic ecosystems, has become a grave problem. The contamination of water by pesticides, either directly or indirectly, can lead to fish deaths, reduced fish productivity, or elevated concentrations of undesirable chemicals in edible fish tissue which can affect the health of humans consuming these fish.

Thiamethoxam is a neonicotinoid insecticide active against a broad range of commercially important sucking and chewing pests.

Glutamate dehydrogenase (GDH, EC 1.4.1.3) is an enzyme present in all the vertebrate tissues and it represents a key link between catabolic and metabolic pathways. The enzyme is known to provide an essential link between carbohydrate and amino acid metabolism, glutamate being a key intermediary in the transfer of amino groups to and from other amino acids (McGivan and Chappell 1975; Williamson et al. 1976; Storey et al. 1978; Wanders et al. 1983). GDH also contributes ammonium ions to the urea cycle (Williamson et al. 1976) and is a distribution point for a-amino functionalities (Schmidt and Schmidt 1988; Brosnan, 2000). Though this enzyme is important in view of physiological action no substantial work has been done in fish and therefore, the present work was carried out to fill out the lacunae.

MATERIALS AND METHODS

Healthy fingerlings of *Ophiocephalus punctatus* measuring the size of 12-15cm and weight 14-18gm were purchased from the Fish market of Rajura. It was carried to the laboratory in hygienic condition and acclimatized for fifteen days. During this period of acclimatization fish were fed with boiled eggs and rice bran. Thiamethoxam LC-50 for 96 hrs was carried out thrice to confirm exact dose and confirmed sub-lethal concentration at 29.483 ppm. Then the fish were divided into six groups. Each group was contained twenty fish each. Later all these fish were exposed to sub-lethal concentration of thiamethoxam for 24, 48, 72 and 96 hrs respectively. Tissues were removed from the fish after decapitation.

The GDH (GDH: L-glutamate, NAD Oxidoreductase, EC 1.4.1.3) activity was determined according to Lee and Lardy, (1965), as modified by Pramilamma and Swami, (1975). The 4% (w/v) homogenate was prepared in 2.25 M sucrose solution. Supernatant of the homogenate obtained by centrifugation at 2500 rpm for 15 minutes was used for enzyme assay. The reaction mixture in a final volume of 2.5 ml contained 50 µmoles of substrate (sodium glutamate), 100 µmoles of phosphate buffer (pH 7.4), 2 µmoles of INT and 0.1 µmoles of NAD. The above mixture was made upto 2 ml with double distilled water. The reaction, in all the samples, was started by the addition of 0.5 ml of crude enzyme extract (equivalent to 20-25 mg of tissue) after half an hour incubation of the samples at 37^{o}C, the reaction was stopped by adding 5 ml of glacial acetic acid. The formazan formed was extracted overnight in 5 ml of cold toluene. The colour intensity of the formazan, proportional to enzymatic activity was read at 495 nm against toluene as a blank. The enzyme activity was expressed as µmoles of formazan/hour/100 mg tissue.

Values were calculated by using software Orgin-50 and figures were prepared with the help of Microsoft Excel and Adobe Photoshop-7.

RESULTS

Glutamate dehydrogenase (GDH) plays an important role in both catabolic and anabolic functions of the cells. It has been observed that the organochloride, thiamethoxam affect greatly the GDH activity in the liver, kidney, gill and muscle of *Ophiocephalus punctatus.*

Liver

Liver is the metabolic center for many biochemical reactions. In the liver of controlled fish the lowest GDH activity (0.428 ± 0.0047µmoles/hour/100 mg liver) was observed for the entire period of 96hrs (**Fig. 1**). Thiamethoxam at sublethal concentration increased the GDH activity from 24hrs to 96 hrs of exposure. GDH activity noted high (0.637 ± 0.0082 µmoles/ hour/100 mg liver) on 4th day of exposure i.e. at 96hrs and lower (0.463 ± 0.0088 µmoles/hour/100 mg liver) at 24hrs of exposure. This continuous increase in enzyme activity in the liver was non-significant. Comparative to all the tissues studied the liver exhibited the maximum GDH activity.

Kidney

Kidney is an organ where the blood is filtered. In the kidney of controlled fish the lowest

GDH activity (0.302 ± 0.0048 µmoles/hour/100 mg kidney) was observed for the entire period of 96hrs. On exposure to thiamethoxam at sub-lethal concentration GDH activity in the kidney was observed to increase subsequently from 24hr to 48hrs. The maximum (0.42 ± 0.0124 µmoles/ hour/100 mg kidney) GDH activity was noted at 96hrs of exposure and lowest (0.315 ± 0.005 µmoles/hour/100 mg kidney) at 24hrs (**Fig. 2**). Sudden downfall (0.337 ± 0.0049 µmoles/hour/100 mg kidney) in GDH activity was noted at 72hr as compared to 48 and 98 hrs of exposure. This increase in GDH activity was non-significant for all the period of exposure.

Gill

It is the organ of aquatic respiration shows dramatic increase in GDH activity from 24hrs to 96 hrs on exposure to sub-lethal concentration of thiamethoxam. In control fish the GDH activity was noted to constant (0.322 ± 0.0048 µmoles/ hour/100 mg gills) upto 96hrs (**Fig. 3**). This increase in GDH activity was non-significant for all the period of exposure. However the maximum GDH activity (0.442 ± 0.0048 µmoles/hour/100 mg gills) was observed at 98hrs of exposure and lowest (0.342 ± 0.007 µmoles/hour/100 mg gills) at 24hrs.

Muscle

In the muscle of controlled fish the GDH activity was 0.347 ± 0.0049 µmoles/hour/100 mg muscles for the whole period of experimentation (**Fig. 4**). It shows continuous increase in GDH activity from 24hrs to 96 hrs on exposure to sub-lethal concentration of thiamethoxam. The increase in GDH activity was non-significant for all the period of exposure. The maximum GDH activity in the muscle was 0.463 ± 0.008 µmoles/hour/100 mg at 98hrs of exposure and at 24hrs of exposure it was lowest i.e. 0.352 ± 0.0098 µmoles/hour/100 mg muscles.

DISCUSSION

Despite the fact that, the glutamate dehydrogenase (GDH) is an important enzyme but still not much work has been done on fishes in response to xenobiotic substances. GDH is a mitochondrial enzyme, catalyses the oxidative deamination of glutamate, providing α-ketoglutarate to the kerbs cycle (Reddy and Venugopal, 1990). This enzyme is having several metabolic functions with great physiological significance. It is closely associated with the detoxification mechanisms of tissues. GDH in extra-hepatic tissues could be utilized for channeling of ammonia released during proteolysis for its detoxification into urea in the liver. Hence, the activity of GDH is considered as sensitive indicators of stress (Gould et al., 1976). Therefore attempt has been made to study the effect of Thiamethoxam on the glutamate dehydrogenase activity in the economically important freshwater snake heads, *Ophiocephalus punctatus*.

The present study revealed that the thiamethoxam provoked alterations in GDH activity and shown non-significant increase in all tissues after exposure to sub-lethal concentration from 24hrs to 96hrs. The successive increase was observed in the GDH activities in all the organs of the *O. punctatus* upon exposure to thiamethoxam. This implies the active transdeamination of amino acids for the incorporation of ketoacids into the

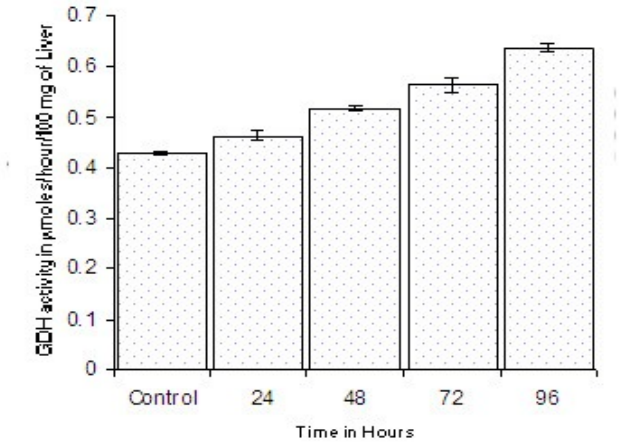

Fig. 1 Showing increase in GDH activity in the liver of *O. punctatus* on exposure to sub-lethal concentration of thiamethoxam for short duration.

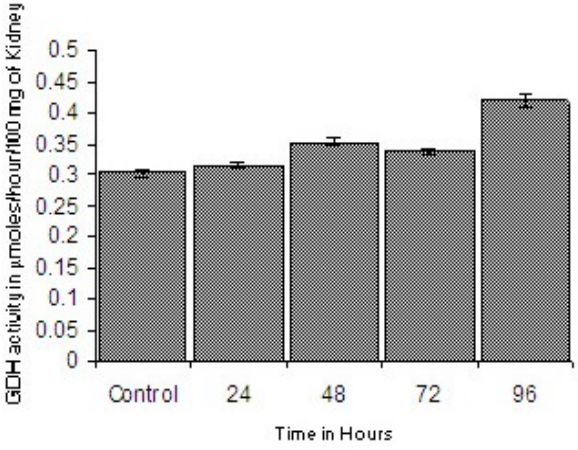

Fig. 2 Showing increase in GDH activity in the kidney of *O. punctatus* on exposure to sub-lethal concentration of thiamethoxam for short duration.

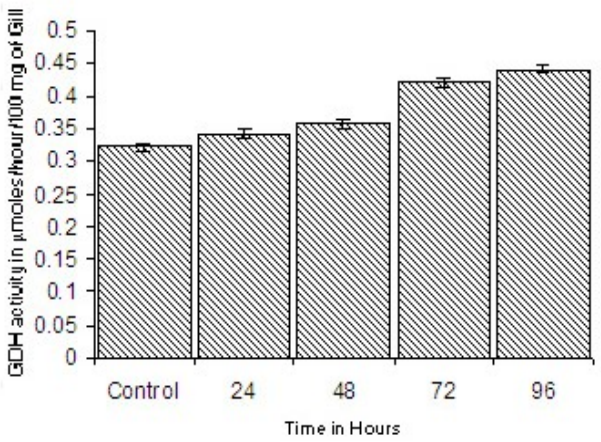

Fig. 3 Showing increase in GDH activity in the gill of *O. punctatus* on exposure to sub-lethal concentration of thiamethoxam for short duration.

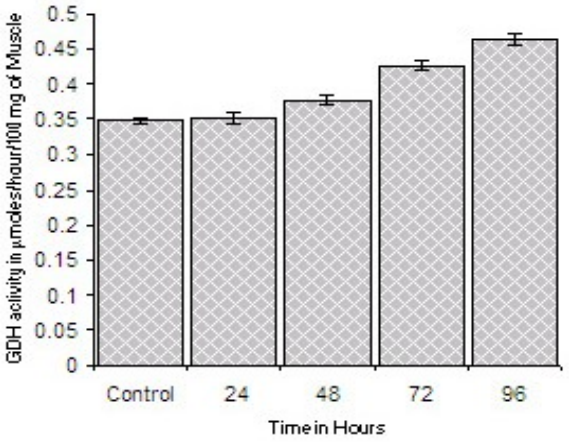

Fig. 4 Showing increase in GDH activity in the muscle of *O. punctatus* on exposure to sub-lethal concentration of thiamethoxam for short duration.

TCA cycle to release necessary energy required for the synthesis of new proteins (Sreedevi et al., 1992; Sivaramakrishna and Radhakrishnaiah, 1998). Subsequent increase in the enzyme specifies the utilization of amino acids. Improvement in GDH activity in the tissues provided ketoglutarate and reduced nucleotides, which may fulfill the energy requirements during toxicity manifestations (Chandravathy and Reddy 1994). However the amino acids appear to be mobilized to get transamination to 2-keto acids, for use in the production of energy rich compounds (David, 1995; Rajmannar and Manohar, 1998; Deva, 2000).

GDH in extra-hepatic tissue like kidney, gill and muscles could be utilized for its ultimate detoxification to urea in the liver. In the present study the significant elevation in activities of these enzymes in the organs of fish exposed to the lethal concentration of cypermethrin indicates greater association of oilgomers of these enzymes in response to toxic stress. This shows that oxidative deamination is contributing higher ammonia production. The high levels of ammonia produced is not eliminated but is salvaged through GDH activity which is utilized for amino acid synthesis through transaminases (David, 1995; Deva, 2000 and Prashanth, 2003).

The steady increase in the activities of GDH leads to metabolic compensation and allow the animal to adapt to the imposed toxic stress. The elevation in GDH activity at the sub-lethal concentration could lead to increased production of glutamate in order to eliminate ammonia. The GDH activity in the present study exhibited a progressive enhancement in all tissues (liver, kidney, gill and muscle), throughout the exposure, suggesting a need for α-ketoglutarate. The regulatory roles of this enzyme as observed in mammalian models in checking the deamination process were reported earlier (Philip et al., 1988; Ramana Rao et al., 1990; Reddy and Venugopal, 1990; Reddy and Yellama, 1991; David, 1995; Deva, 2000 and Shobha Rani et al., 2001).

CONCLUSION

It has been concluded that thiamethoxam at sub-lethal concentration may alter the glutamate metabolism in all the tissues by enhancing the GDH activity and for the evaluation of its effect further study also requires in this direction.

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