Int J Pharma Res Health Sci. 2018; 6 (1): 2165-68
DOI:10.21276/ijprhs.2018.01.14
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CODEN (USA)-IJPRUR, e-ISSN: 2348-6465

PHS Scientific House
PHARMAHEALTHSCIENCES

International Journal of Pharma Research and Health Sciences

Available online at www.pharmahealthsciences.net

Original Article

Effect of Ethion (50%EC) on Enzymatic Activities of AAT and ALAT in Freshwater Fish *Labeo rohita* (Hamilton)

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ARTICLE INFO

Received: 03 Jan 2018
Accepted: 12 Feb 2018

ABSTRACT

The ability to predict the effects of pollutants or chemicals on organisms and to extrapolate toxicant effects from laboratory to population and community levels has become a very important factor. The Indian major carp *Labeo rohita* freshwater fish were exposed to lethal (1.2ppm/l) and sub-lethal (0.12ppm 1/10th of lethal) concentrations for 5th, 10th and 15th days of Ethion (50%EC) to examine the enzymatic activity , in different tissues like gill, liver, kidney, brain and muscle and control group were also maintained in all the exposure periods. Alanine aminotransferase (ALAT) and Aspartate aminotransferase (AAT) are liver specific enzymes which are more sensitive measure of hepatotoxicity and histophathalogical changes. The activity levels of AAT and ALAT were increased in all tissues of sublethal concentrations compared with controls when exposed in, 5th, 10th and 15th day. The AAT activity levels in the control fish were in the order of: Brain<Gill< Kidney<Muscle<Liver and Sublethals: Brain<Gill< Kidney<Muscle<Liver. The activity levels of ALAT in the control fish were in the order of: Liver > Brain > Muscle > Kidney > Gill and Sublethals: Liver > Muscle > Kidney > Gill >Brain.

KEYWORDS: *Labeo rohita*, sub-lethal concentration, Ethion, AAT and ALAT.

1. INTRODUCTION

Aquatic organisms are sensitive to pesticide chemicals, and toxic concentrations may rise not only from excessive spillage of agriculture practices but also from several other sources. Apart from causing death either directly or due to starvation by destruction of food organisms, many pesticides with the evidence of tissue damage. Ideally pesticides should be highly selective, destroying target organisms while

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leaving non- target organisms unharmed Ernest Hodgson, [1]; David Hernandez-Moreno *et al.*, [2]. The improper management of pesticides in agriculture crops could result in contamination of water bodies (Candida Toni *et al.*, [3]; Capkin and Altinok, [4]. The pesticide stress was known to induce significant change in protein metabolism; it is likely that the amino transferases were also considerably affected. Increased activities of AAT and ALAT in different tissues of fish either increased operation of transamination or increased synthesis of amino acids from other sources like glucose of fatty acids during Ethion.

2. MATERIAL AND METHODS

Healthy freshwater fish, *Labeo rohita* (Hamilton) size [6±7 cm total length (TL) and 6.5±7.5 g body weight] were collected from the fish farm, Kuchipudi, Guntur District of A.P, India. Then the fish was acclimatized to the laboratory conditions with sufficient dechlorinated water for 10 to15 days at room temperature 28±2^{0}C. The fish were fed with fish meal, rice and commercial fish pellets once in two days, at the same time water was renewed every day rich in oxygen (aeration) and feeding was stopped one day prior to the experimentation. Ethion (50%EC) is a broad-spectrum pesticide used to control insects, pest, and mites in agricultural field. The stock solution of the toxicant was prepared in one liter of 100% pure acetone. Concentration of Ethion was found in 96 hr 1.2ppm/l as a lethal and 1/10th was taken as a sublethal concentration along with control group was maintained for each experiment. The activity of SGOT/AAT and SGPT/ALAT was determined by the method of Reitman and Frankel 5. The selected tissue was homogenized in 5% ice-cold 0.25 M sucrose solution. The supernatants were used for the analysis of the enzyme activities. The fish were exposed to lethal and sublethal concentrations in different tissues like gill, liver, kidney, brain and muscle for, 5th, 10th and 15th days to determine the enzyme activity levels.

3. RESULT

The calculated values of AAT & ALAT and percent change over control along with standard deviations were given in Table 1 to 2 and graphically represented in Fig 1 to 2. The changes in the levels of AAT and ALAT were studied in different tissue of brain, liver, muscle, gill and kidney in the test fish *Labeo rohita* under sub-lethal and lethal concentrations of Ethion (50%EC) after 5th, 10th and 15th day's exposures. The values were expressed as IU/L of pyruvate formed /mg protein /h.

Under 5th day sub lethal concentration of Ethion (50%EC), the AAT activity was increase in all the tissues of test fish, maximum increased was in liver (17.66%) and minimum increased in brain (9.42%) increased in the series of Brain<Gill<Kidney<Muscle<Liver. Under sublethal exposure to Ethion for 10th day, the activity of AAT was found to increased in all the tissues of test fish and maximum increase was in liver (23.61) minimum increase (13.87%) in brain, increased in the series of Brain<Gill< Kidney<Muscle<Liver. Under 15th day sublethal exposure to Ethion was found to increase in all the tissues of test fish, maximum increased was in liver (28.72%) and minimum increased in brain (16.75%) increased in the series of Brain< Kidney<Gill<Muscle<Liver.

The ALAT specific activity levels increased significantly during 5, 10 and 15th days exposure period to compare with controls. Under 5th day sublethal exposure of Ethion maximum percentage of elevation was in muscle (34.02) and minimum percentage of elevation was in brain (17.59) increased in the series of Brain<Kidney<Gill< Liver< Muscle. Under Ethion 10days sublethal exposure, maximum percentage of elevation in ALAT activity was in liver (43.50) and minimum elevation was in brain (24.42) increased in the series of Brain<Gill<Kidney<Muscle <Liver. Under Ethion 15 days sublethal exposure period, maximum percentage of elevation in ALAT activity was (38.94) in liver and minimum elevation was (21.68) in brain in the series of Brain< Gill<Kidney<Muscle<Liver.

Table 1: Changes in the specific activity levels of Aspartate aminotransferase (AAT) (µ moles of formazan /mg protein/hr) and percent change over control in different tissues of the freshwater fish, *Labeo rohita* exposed to sub-lethal and lethal concentrations of Ethion (50%EC)

Tissues	Control (mg/g)	5 Days Sublethal (mg/g)	% Change	10 Days sublethal (mg/g)	% Change	15 days sublethal	% Change
Muscle	6.80 ±0.10	7.82 ±0.07	+16.27	8.10 ±0.04	+19.11	8.23 0.05	+21.02
Gill	5.2 ±0.05	5.90 ±0.02	+11.95	6.13 ±0.13	+16.32	6.40 ±0.12	+19.54
Liver	4.70 ±0.20	5.53 ±0.05	+17.66	5.81 ±0.05	+23.61	6.05 ±0.08	+28.72
Kidney	4.05 ±0.15	4.66 ±0.09	+15.06	4.78 ±0.02	+18.02	4.83 ±0.15	+19.25
Brain	3.82 ±0.06	4.18 ±0.04	+9.42	4.35 ±0.01	+13.87	4.46 ±0.20	+16.75

Values are the mean of five observations ;(±) indicates the standard deviation:
Values are significantly at *P*< 0.05

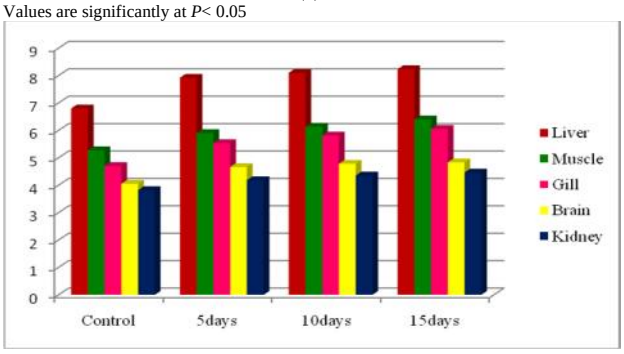

Fig 1: Changes in the specific activity levels of Aspartate aminotransferase (AAT) (µ moles of formazan /mg protein/hr) and percent change over control in different tissues of the freshwater fish, *Labeo rohita* exposed to sub-lethal and lethal concentrations of Ethion (50%EC)

Table 2: Changes in the specific activity levels of Alanine aminotransferases (ALAT) (µ moles of formazan /mg protein/hr) and percent change over control in different tissues of the freshwater fish, *Labeo rohita* exposed to sub-lethal and lethal concentrations of Ethion (50%EC)

Tissues	Control (mg/g)	5 days Sublethal (mg/g)	% Change	10days Sublethal (mg/g)	% Change	15days Sublethal (mg/g)	% Change
Gill	5.40 ±0.07	6.74 ±0.08	+24.81	7.10 ±0.02	+31.48	6.92 ±0.05	+28.14
Brain	4.15 ±0.11	4.88 ±0.02	+17.59	5.16 ±0.08	+24.42	5.05 ±0.12	+21.68
Kidney	5.10 ±0.08	6.29 ±0.06	+23.33	6.74 ±0.02	+32.15	6.58 ±0.09	+29.02
Liver	3.54 ±0.34	4.70 ±0.01	+32.76	5.08 ±0.05	+43.50	4.92 ±0.23	+38.94
Muscle	4.32 ±0.10	5.79 ±0.05	+34.02	5.94 ±0.09	+37.50	5.84 ±0.15	+35.18

Values are the mean of five observations ;(±) indicates the standard deviation:
Values are significantly at $P< 0.05$

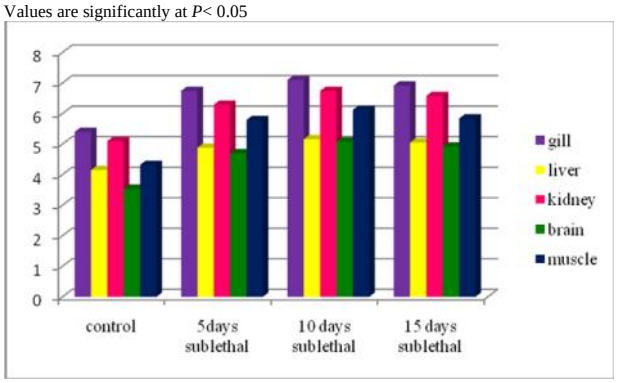

Fig 2; Changes in the specific activity levels of Alanine aminotransferases (ALAT) (µ moles of formazan /mg protein/hr) and percent change over control in different tissues of the freshwater fish, *Labeo rohita* exposed to sub-lethal and lethal concentrations of Ethion (50%EC)

4. DISCUSSION

AAT catalyses reversible transamination of glutamate and oxalo acetate to α-ketoglutate and aspartate, while ALAT catalyses the reversible transamination of glutamate and pyruvate to α-keto glutamate and alanine. Thus, the aminotransferases along with GDH contribute some strategic substances such as α-ketoglutarate, pyruvate, oxaloacetate, glutamate etc., to oxidative metabolism Prashanth and Neelagoud, 6. Pesticides also influence the activity of different enzymes. A slight variation in enzyme activities would affect the organism Tilak *et al.,* 15. Aminotransferases mobilize the aminoacids into carbohydrate and lipid metabolism. There exists a rapid turnover of free amino acids into carbohydrate and lipid circulating fluids and utilize for various purposes through introversions Holi *et al.,* [16].

The increase in activities of aminotransferases as observed in the present study were in agreement with earlier reports, demonstrating a consistent increase in the activities enzymes under conditions of enhanced gluconeogenesis. In the present study, pesticides increase the activities of ALAT enzyme and increase the activities of AAT, while the activities of a few enzymes remain unchanged in various tissues of fish *Labeo rohita* in 5th, 10th and 15th days of lethal and sublethal concentrations. The elevation of AAT activity provides the oxaloacetate require for the gluconeogenesis pathway to meet the additional supply of glucose for the production of energy under reduced phase of oxidative metabolism. Elevation in the levels of AAT in different tissues of brain, muscle, gill and kidney of the fish *L.rohita* can be ketoacids like α-ketoglutarate and oxaloacetate for contributing to gluconeogenesis and or energy production necessary to meet the energy demand under the toxic manifestations. The alterations in the levels of aminotransferases induce by Ethion clearly indicate that stress bring about the metabolic reorientation in the tissues by raising energy resources through trasaminases systems.

Aminotransaminases play an important role in the utilization of amino acids for the oxidation and gluconeogenesis Kumar *et al.,* [7]. The elevation of AAT activity provides the axaloacetate required for the gluconeogenesis pathway to meet the additional supply of glucose for the production of energy under reduced phase of oxidative metabolism Velumurugan *et al.,* [8]. The elevation in ALAT activity measured in liver of eel tissues during exposure to propanol due the existence of heavy drain on metabolites during propoil stress to provide inter mediates to the kreb's cycle Sancho *et al.,* [9]. Similar increase in trasaminase enzymes have been reported in fish tissues exposed to pesticides David *et al.,* [10]. This group of pesticides interferes with the process of synaptic transmission by inhibiting the activity of acetylcholinesterase. Its effects on nervous system are well known through the inhibition of the acetylcholinesterase enzyme, which plays an important role in neurotransmission at cholinergic synapses by rapid hydrolysis of neurotransmitter acetylcholine into choline and acetate. The measurement of transaminase activities in serum is frequently used to diagnostic tool in human and animals Barse *et al.,* [11].

According to Hori *et al.,* [16], phenol and its derivatives can alter the protein metabolism by altering transamination rate of amino acids by enhancing the activity of ASAT and ALAT. Significant increase in the activity of alanine aminotransferase (ALAT) in brain, liver and kidney of *Channa punctatus* for 96 hr due to cypermethrin stress Kumar *et al.,* [7]. De la Torre *et al.,* [2] reported elevation in levels of trasaminases ALAT and AAT in fish *Cnesterodon decemmaculatus using* those enzymes as biomarkers of polluted water. The significant increase in the activities AAT and ALAT in different tissues exposure of cypermethrin due to incorporation of ketoacids into the TCA cycle via generation of glutamate through tissue transamination followed by their conversion of α-ketoglutarate through oxidative deamination to favors gluconeogenesis or energy production Prasanth and Neelagund, *et al.,* [6]. Sancho *et al* [9] reported an elevation of AAT and ALAT activities in eel *Anguilla Anguilla* under propanol intoxication.

5. CONCLUSION

AAT and ALAT are located in both mitochondrial and cytosal fractions of the cell. A close relation appears to exist between the mitochondrial integrity and transaminase levels and any modification in the organization of mitochondria is bound to alter the enzyme systems associated with it. The AAT activity levels was increased levels was shown in 5, 10 and 15th days of sublethals. The ALAT activity levels increased in sublethals was observed in the present study may also be due to the mitochondrial disruption and damage as a result of Ethion induced stress.

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Conflict of Interest: None

Source of Funding: Nil