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IMPACT ON THE ASPARTATE AND ALANINE TRANSAMINASES OF *LABEO ROHITA* (HAMILTON) AFTER THE ACUTE AND CHRONIC EXPOSURE OF BIOPESTICIDE NEEMPLEX (NEEM BASED PESTICIDE)

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ABSTRACT

In the present investigation, the liver of Labeo rohita was selected for the estimation of AST and ALT activity after the acute (4 days) and chronic exposure (30 days) of biopesticide Neemplex. The 96h LC_{50} value determined by Finney's Probit Analysis Method (1971) was found to be 47.32 ppm. For biochemical estimation, three groups were selected during the acute and chronic toxicity tests in which the group first was taken as the control group (no biopesticides used) and the other two groups were given sub lethal concentrations of Neemplex. The group II was treated with 1/15th (=3.154ppm) concentration of 96hr LC_{50} value of Neemplex and the group III was treated with 1/10th (=4.732ppm) concentration of Neemplex. These results were later compared to the Group I which was taken as control. The results revealed that there was a significant increase in the AST and ALT activity in the liver homogenate of treated fishes as compared to the control group. The elevated levels of AST and ALT may be due to hepatotoxicity of liver cells. Aminotransferases are the biomarkers to know the intensity of liver damage. From the present study, it may be concluded that the analysis of enzyme activity of fishes can effectively be used as an indicator of fish health.

Key words: *Labeo rohita,* Neemplex, Liver, AST, ALT, hepatotoxicity.

INTRODUCTION

Enzymes are biochemical molecules that control metabolic processes of organisms, thus a slight variation in enzyme activities would affect the organism (Roy, S.S, 2002). Biochemical changes induced by pesticidal stress lead to metabolic disturbance, vital enzymes inhibition, retardation of growth and reduction of fecundity and longevity of organisms (Fatima *et al*., 2006). When the integrity of a cell is disrupted, enzymes escape into plasma/serum, where their activity can be measured as a useful index of cell integrity (Coppo *et al.*, 2002). Thus, by estimating the enzyme activities in an organism, we can easily identify disturbance in its metabolism. Liver is an established organ in fishes and plays an important role in uptake, accumulation, biotransformation and excretion of xenobiotics (Thophon *et al*., 2003). The use of biochemical measurements in organisms as indicators of pollution, give information about the adaptive or deleterious responses in organisms exposed to a certain amount of chemicals. Such analysis provides early warning signals before other toxicological points, including death are evident (Livingstone, 1998). Pesticides are known for the alteration of enzyme activities in living organisms. They disturb the biochemical processes, leading in some cases to fatal results. In general, pesticides attack the active sites of enzymes, inhibiting essential enzyme function. Alterations in enzyme activities have been widely used in the determination of effects caused by various contaminants in aquatic ecosystems. In the present study, the effect of biopesticide Neemplex on the aspartate and alanine transaminase activities in the liver tissues of *Labeo rohita* was determined. Neemplex is a botanical insecticide made from the seed kernels of the neem. The formulation contains a minimum of 0.15% EC (1500PPM) of azadirachtin, the most bioactive compound of neem. Neem (*Azadirachta indica* A. Juss) is a traditional and highly esteemed medical tree for the people of Indian sub-continent. Biological activities and medical properties of neem have been extensively reviewed by Biswas *et al*. (2002). Azadirachtin (a tetranotriterpenoid) is one of major components (Kraus *et al*., 1981; Broughton *et al*., 1986; Saxena, 1990) of neem, which have pesticide property (Anjaneyulu and Mishra, 1998).

Aspartate transaminase (AST), also called aspartate aminotransferase (AspAT/ASAT/AAT) or serum glutamic oxaloacetic transaminase (SGOT) is a pyridoxal phosphate (PLP)-dependent transaminase enzyme. It catalyzes the reversible transfer of an α-amino group between aspartate and glutamate and, as such, is an important enzyme in amino acid metabolism. AST is found in the liver, heart, skeletal muscle, kidneys, brain, and red blood cells, and it is commonly measured

clinically as a marker for liver health. Aspartate transaminase catalyzes the interconversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate.

Alanine transaminase or ALT is another transaminase enzyme. It is also called serum glutamic pyruvic transaminase (SGPT) or alanine aminotransferase (ALAT). ALT is found in serum and in various bodily tissues, but is most commonly associated with the liver. It catalyzes the transfer of an amino group from alanine to a-ketoglutarate, the products of this reversible transamination reaction being pyruvate and glutamate. It is commonly measured clinically as a part of a diagnostic evaluation of hepatocellular injury, to determine liver health.

Biopesticides are usually considered as less toxic to non-target species and so little attention has been paid to assess the toxicity of them against fishes and other non-target animals. Since fishes are the top consumers of aquatic ecosystem and thus chances of pesticide bioaccumulation are greater in them. Fishes act as bioindicators of aquatic pollution. In the present study, *Labeo rohita* has been selected as a test model animal because of its easy availability, high food value and wide distribution. *Labeo rohita* belongs to family cyprinidae of class teleostomi (Osteichthyes) and is the most popular food fish in India.

MATERIALS AND METHODS

The experiments of the present study were performed at Department of Zoology Government Science and Commerce College Benazir Bhopal (M.P). Glass aquaria of the size 24"×12"×18" were set up in the laboratory. All the aquaria were of the capacity of 60 liters. Aquaria were provided with all the necessary equipments such as aerators, artificial light, facial matter extraction tube and water removing pipes to maintain the natural possible conditions for the test organism. The experimental fish, *Labeo rohita* was obtained from Patra fish farm Barkhedi Bhopal (M.P). They weighed 50g±2g and their length was in the range of 12cm±2. They were brought to laboratory carefully in oxygen filled polythene bags in card board boxes to avoid any injury. They were disinfected by giving a bath for five minutes in $KMnO_4$ solution. Thereafter, they were transferred to glass aquaria filled with dechlorinated water. The fishes were acclimated to the laboratory conditions for 20 days prior to the experiment. During acclimatization fishes were fed daily with commercial fish food which was given at morning hours. Water was replaced every 24h after feeding in order to maintain a healthy environment for the fish during acclimatization period. This ensures sufficient oxygen supply for the fish and the environment

was devoid of any accumulated metabolic wastes. Dead fishes whenever located were removed immediately to avoid the fouling of the water. Prior to conducting the bioassay for biochemical examination, a toxicity bioassay was run in the same water to estimate the 96hr LC_{50} value of Neemplex for *Labeo rohita* and the same was found to be 47.32 ppm determined by Finney's Probit Analysis Method (1971). In the present study, two sublethal concentrations (1/15th and 1/10th of LC_{50} values) of Neemplex were prepared and induced to *Labeo rohita* for 4 days (acute exposure) and 30 days (chronic exposure). Three groups were selected during the toxicity tests in which the group first was taken as the control group (no biopesticides used) and the other two groups were given sub lethal concentrations of Neemplex. The group II was treated with $1/15^{th}$ concentration of 96hr LC_{50} value of Neemplex and the group III was treated with $1/10^{th}$ concentration of 96hr LC_{50} value of Neemplex. Six fishes were used in control as well as in experimental batches.

The estimation of two important amino transferase enzymes (AST and ALT) was done in the present study. To study the activity of aspartate transaminase and alanine transaminase, homogenate of liver tissue from control and experimental fishes was prepared. For the preparation of homogenate, fishes were dissected and the vital organ liver was removed, cleaned and homogenized in 0.25M ice cold sucrose solution. Homogenates were then centrifuged for 15 minutes at 25000 rpm in cooling centrifuging machine and clear supernatants were used as a source of enzyme. The enzyme extracts were kept at $0^{\circ}C$ until required. Aspartate transaminase (AST), also called aspartate aminotransferase (AspAT) or serum glutamic oxaloacetic transaminase (SGOT) is a pyridoxal phosphate (PLP)-dependent transaminase enzyme. It catalyzes the reversible transfer of an α-amino group between aspartate and glutamate and, as such, is an important enzyme in amino acid metabolism. It is commonly found in the liver, heart and skeletal muscles and it is commonly measured clinically as a marker for liver health. It catalyzes the interconversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate. Its activity is expressed in units per litre (U/L) homogenate. Unit (U) is an international unit of enzyme activity, which is the amount of enzyme required to catalyze the conversion 1µmol of substrate to product in 1 minute. Therefore 1 U/L = 1µmol/min/L.

Alanine transaminase or ALT is another transaminase enzyme. It is also called serum glutamic pyruvic transaminase (SGPT) or alanine aminotransferase (ALAT). It is found in serum and in various bodily tissues, but is most commonly associated with the liver. It catalyzes the transfer of

an amino group from alanine to a-ketoglutarate, the products of this reversible transamination reaction being pyruvate and glutamate. It is commonly measured clinically as a part of a diagnostic evaluation of hepatocellular injury, to determine liver health. Its activity is also expressed in units per litre (U/L) homogenate. The activity of these two enzymes in the liver was estimated by using the method of Reitman and Frankel (1957). The products formed by enzyme action are glutamate and oxaloacetate for AST and glutamate and pyruvate for ALT. Oxaloacetate formed in the AST is unstable and immediately converted into pyruvate. Hence, pyruvate standard was used in both enzyme estimations.

RESULTS

Estimation of enzyme activities in the tissue and organs of aquatic organisms is one of the emerging areas in toxicological monitoring and remediation programs (Oluah *et al*., 2005). Enzymes are commonly used for detecting or diagnosing physiological changes in fish exposed to various toxic substances. Since the presence of the toxicant in water has been found to alter the physiology and biochemistry of fish, therefore, there was a need to examine the enzymatic changes associated with Neemplex biopesticide under laboratory conditions. In the present study, liver of *Labeo rohita* was selected for the estimation of AST and ALT activity. Data were summarized by different statistical methods.

During acute exposure of Neemplex to *Labeo rohita* for 4 days, the AST and ALT activity in the liver was estimated. Two sublethal concentrations of $1/15^{th}$ (=3.154 ppm) and $1/10^{th}$ (=4.732 ppm) of 96hr LC_{50} values of Neemplex were first prepared and then induced to Group II and Group III respectively. These results were later compared to the Group I which was taken as control. Six fishes were present in each group. The results revealed that there was a significant increase in the AST and ALT activity in the liver homogenate of treated fishes when compared to the control group **(Table1).** During chronic exposure, the fishes were first exposed to two sublethal concentrations of $1/15^{th}$ (=3.154 ppm) and $1/10^{th}$ (=4.732 ppm) of 96hr LC_{50} values of Neemplex for 30 days. These results were later compared to the Group I which was taken as control. The results revealed that there was a significant increase in the AST and ALT activity in the liver homogenate of treated fishes when compared to the control group **(Table 2).**

S.NO.	GROUPS	CONC. OF NEEMPLEX (ppm)	AST ACTIVITY (IUL^{-1})	PERCENT CONTROL (%)	ALT ACTIVITY (IUL^{-1})	PERCENT CONTROL (%)
1	GROUP I (CONTROL)	0.000	60.58±5.24	100	52.40±6.25	100
2	GROUP II	3.154	65.20±3.91*	107.62	56.72±4.34*	108.24
3	GROUP III	4.732	72.52±4.35*	119.70	63.16±5.12*	120.53

Values are given as means ± Standard deviation for six fish in each group,*significant at $p < 0.05$.

TABLE 1: Showing changes in the levels of AST and ALT activity after the exposure of $1/15^{th}$ and $1/10^{th}$ of 96hr LC_{50} value of Neemplex for 4 days.

S.NO.	GROUPS	CONC. OF NEEMPLEX (ppm)	AST ACTIVITY (IUL^{-1})	PERCENT CONTROL (%)	ALT ACTIVITY (IUL^{-1})	PERCENT CONTROL (%)
1	GROUP I (CONTROL)	0.000	63.42±5.20	100	53.45±6.26	100
2	GROUP II	3.154	70.08±3.34*	110.50	62.88±5.72*	117.64
3	GROUP III	4.732	82.40±4.20*	129.92	71.40±5.60*	133.58

Values are given as means ± Standard deviation for six fish in each group,*significant at p < 0.05.

TABLE 2: Showing changes in the levels of AST and ALT activity after the exposure of 1/15th and 1/10th of 96hr LC_{50} value of Neemplex for 30 days.

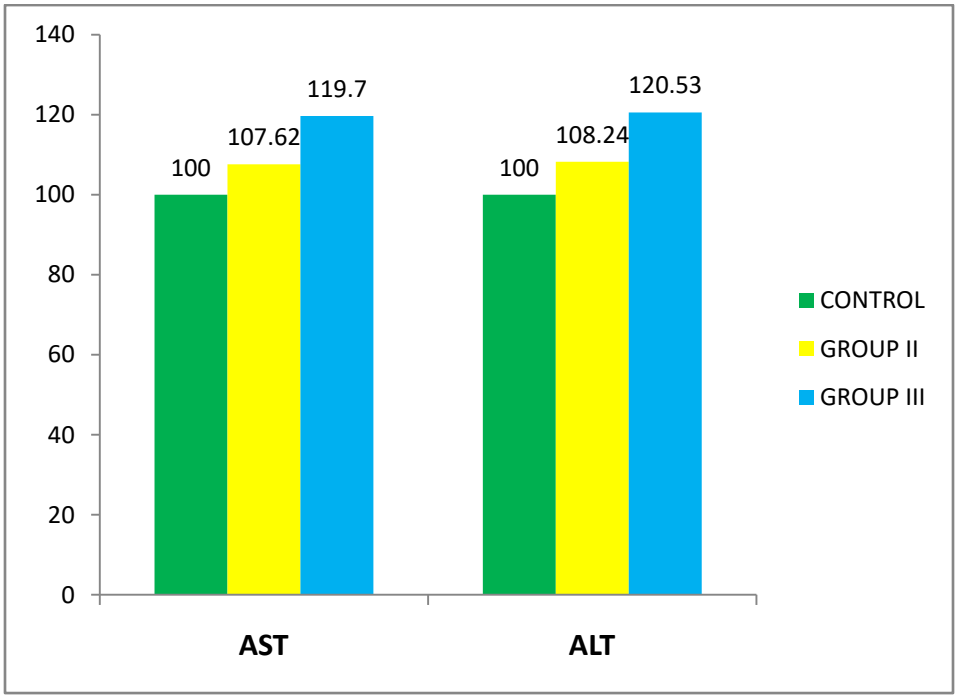

CHART NO. 1: Showing the comparison between the changes in AST and ALT levels of liver in Neemplex treated fish, *Labeo rohita* during the acute exposure of 4 days.

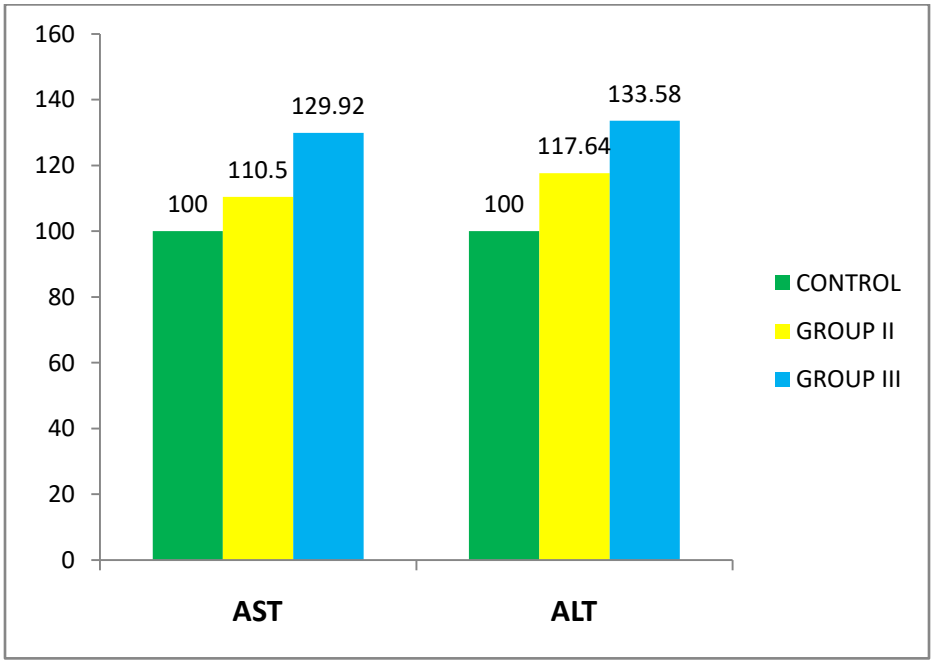

CHART NO. 2: Showing the comparison between the changes in AST and ALT levels of liver in Neemplex treated fish, *Labeo rohita* during the chronic exposure of 30 days.

DISCUSSION

In the present study the activities of two enzymes aspartate transaminase (AST) and alanine transaminase (ALT) in the liver of Neemplex induced *Labeo rohita* was studied. In acute (4 days) as well as in chronic exposure (30 days), the activities of both the enzymes show a significant increase in the exposed fishes as compared to the normal (control) ones. The alterations were found to be time and dose dependant. Many authors had earlier reported similar findings i.e., increase in AST & ALT activity of fish liver after the exposure of biopesticides and synthetic pesticides. Tiwari and Singh (2009) reported the effect of *Nerium indicum* on AST and ALT in the liver tissue of *Colisa fasciatus*. Significant increase in the level of both the enzymes was noticed after the exposure of fish with the ethanolic extract of *Nerium indicum*. The alterations were found to be time and dose dependant. Saravanan *et al*., (2010) studied the alterations in the AST and ALT enzymes of liver of *Labeo rohita* exposed to endosulfan. As a result of the insecticidal stress, the enzymes AST and ALT revealed significant alterations. Susan *et al*., (2010) also investigated the alterations in the AST and ALT of *Labeo rohita* and *Cirrhinus mrigala* exposed to fenvalerate technical grade. The alterations in the ALT and AST activity in the liver of *Catla catla* exposed to sub-lethal concentration of Cypermethrin was reported by Vani *et al*., (2012). The activities of ALT and AST were significantly enhanced by 30.16 % and 32.47 %, respectively, in comparison with the control group. The alterations in the AST and ALT activity in the liver of the studied fish are in agreement with those observed above by many investigators.

Transaminases play an important role at the junction between the carbohydrate and protein metabolism by interconverting the strategic compounds viz; ketoglutarate, pyruvate and oxaloacetate on one hand and alanine, aspartate and glutamate on the other hand. The increased transaminase activity might be due to increase in transamination reaction i.e. transferring of NH_2 group from amino acid to a ketoacid. Documented evidences showed that transamination and transdeamination reactions are prominent under stress condition (Rajender *et al.*, 1986). Normally, elevation of ALT and AST activities reflects hepatic disease because of its biological location (Ayalogu *et al*., 2001). In the present study, the increase in AST and ALT activity in experimental fishes as compared to control group after acute and chronic exposure of sublethal concentrations of Neemplex might be as a result of hepatocellular damage caused by Neemplex toxicity. This study illustrates that the Neemplex is highly toxic to the fish, *Labeo rohita* and the

stress responses showed by fish are dependent on concentration and duration of exposure. The elevated levels of AST and ALT may be due to hepatotoxicity of liver cells. Aminotransferases are the biomarkers to know the intensity of liver damage. From the present study, it may be concluded that the analysis of enzyme activity of fishes can effectively be used as an indicator of fish health. Long term exposure of organisms to biopesticides means a continuous health hazard for the population. So, human population is at high risk by consuming these toxicated fishes. It is also suggested that these types of toxicological studies are required to monitor the aquatic life and predict the toxic effect of biopesticides on aquatic organisms particularly fish.

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