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IMPACT OF SELECTED TOXICANTS ALONG WITH ACTIVATED CHARCOAL ON ALKALINE PHOSPHATASE ACTIVITY IN SOME TISSUES OF FRESHWATER FISH *LABEO ROHITA*

Lalitha Vinnakota, Nirmala K, Anitha A and Dr.V.Venkata Rathnamma

DEPARTMENT OF ZOOLOGY AND AQUACULTURE

ACHARYA NAGARJUNA UNIVERSITY, GUNTUR-522510. A.P, INDIA

ABSTRACT:

This study determined the effects of azoxystrobin, Copper and their combine synergism along with AC, on the alkaline phosphatase activity of freshwater fish *Labeo rohita*. The fishes were exposed to 1 day lethal, 1 and 14 days sublethal (1/10th of lethal) doses of selected toxicants in static renewal test without and with activated charcoal (AC). Under 1 day lethal exposure the maximum and minimum percentage of augmentation was observed in the intestine (+43.37) and Liver (+1.93) of Combine synergism+ AC and Azoxystrobin+ AC exposures. After 14 days sublethal exposures among selected toxicants combine synergism showed significant decreasing trend while compared with control and 1 day sublethals. Combine synergism was more effective than individual exposures. During the experimental period, the Alkaline phosphatase significantly increased among AC treatment groups at all the exposure periods, with significant differences ($p<0.05$).

Key words: Azoxystrobin, Copper, Combine synergism, Alkaline phosphatase and *Labeo rohita.*

I. INTRODUCTION:

Fish ingest of the pesticides, usually through their gills and mouth. They diffuse into their organs and adipose tissues and causes severe alterations in the tissue biochemistry and histology of fish. In this nature every animal has its own detoxification mechanism to remove foreign substances which are entering their body such as pesticides, heavy metals and pharmaceutical substances. Though, fish is considered as the most important and vital link in the food chain and food web of ecosystem, so understanding of pesticide effects on fish would be really vital for fish conservation. Hence, they called as indicators of toxicants in aquatic bodies. They are more vulnerable and sensitive to toxicants. They respond to exposed toxicants by altering physiological, biological and behavioural functions.

Many toxicants have been reported to produce a number of biochemical changes in fish even at sublethal levels due to changes occurs in ion concentrations, endocrinal activity and enzyme activity. There are numerous toxicants they can form a bound at active site of enzyme and may impair substrate binding activity of enzyme. Enzyme analysis is widely used for rapid detection to predict early warning of pesticide toxicity (Gabriel *et al.*, 2012). Analysis of phosphatase activities is one of the important tools to evaluate toxicity stress of chemicals in aquatic organism. Alkaline phosphatase is a multifunctional and group of enzyme (McComb *et al.*, 2013). Alkaline phosphatase is a glycoprotein of plasma membrane found in all body tissues, primarily liver, bile ducts and bone. Normally the plasma membrane act's as first barrier for toxicants. It is optimally active at alkaline pH environments and present in different isoforms in different tissues with different physicochemical properties. They divided into four isozymes depending upon the site of tissue expression that are intestinal ALP, placental ALP, Germ cell ALP and tissue nonspecific alkaline phosphatase or liver/bone/kidney ALP (Sharma *et al*., 2014). It is helpful in numerous fish activities including osmoregulation, membrane transport, bone formation (Sullip *et al*., 2006), metabolism of carbohydrate, nucleotides and phospholipids. Any damage that occurs to plasma membrane results in alternation of alkaline phosphatase activity (Mohammed 2013). Alkaline Phosphatase (ALP) is hydrolytic lysosomal enzyme and has a role in certain detoxification function and any change in their activities can affect the metabolism of the fish.

II.MATERIALS AND METHODS:

A. **Test fish:** *Labeo rohita*

B. Test chemicals:

i). Azoxystrobin

ii). Copper

iii). Combine synergism

Azoxystrobin, a fungicide and Copper is a heavy metal also use in manufacture of some fertilizers, their mixture might create serious threat to the environment as well as target and non-target organisms like aquatic and terrestrial animals. The LC_{50} of Azoxystrobin (3.3mg/l), Copper (2.9mg/l) individually and combine (1.75mg/l) was calculated by Lalitha and Venkata Rathnamma, 2021. Sublethal concentrations were derived from 96 h LC_{50} as per the procedure given by APHA (2005) to observe various responses of the test fishes on prolonged exposure to selected toxicants.

Fishes with same weight were acclimatized for about 15 days before the commencement of the experiment. They were fed on rice bran which was given daily at morning hours. In the present study $1/10^{th}$ of the 96 h LC_{50} were selected as sublethal concentration and the fishes were exposed to this concentration for a period of 1 and 14 days with and without activated charcoal (AC). A control and positive control batch corresponding to each test group was simultaneously experimented to compare the toxicant values of ALP activity, which was estimated by the method of Mohan and Cook, 1957. The dosage of AC is determined either by the amount of toxin ingested if known or by the body weigh of the person concerned. When the amount of toxin to be bound is known 10 times more AC should be given (Oslon 2010). Hence, in the present study I have given 10 times more AC according to the fish body weight to maintained balanced dosage in all the AC exposed containers. Fresh concentrations were supplied daily to maintain a constant toxic media. At the end of each exposure period, fishes were sacrificed and tissues, such as gill, intestine, liver and kidney were dissected and used for the analysis.

Estimation of Alkaline phosphatase:

The 2% homogenates of the chosen tissues were prepared in 0.25m ice cold sucrose solution and centrifuged at 1000rpm for 20 minutes. The supernatant served as the enzyme source. The reaction mixture of 3.5ml contains 3ml of glycine-NaoH buffer (pH 10.5), 0.1ml of $Mgcl_2$ and 0.3ml of p-nitrophenyl phosphate. The contents were incubated at 37oC for 30 minutes. In alkaline pH buffer system alkaline phosphatase hydrolyses p-nitrophenyl phosphate to pnitrophenol and phosphate. The p-nitrophenol liberated, formed yellow colour at alkaline pH, it has strong absorbance at 410nm. The addition of 9.5ml of 0.085 N NaoH inhibits the reaction. Zero time controls were maintained by adding 0.3ml of P-nitrophenol phosphate prior to the addition of homogenate. The intensity of colour developed was read at 410nm against a reagent blank in a spectrophotometer. The activity was expressed as μ moles of p-nitrophenol produced/min (Tilak *et al.*, 2009).

Statistical analysis:

The results were presented as mean ± SE of three replicates for the first trial and mean ± SE of two replicates for the second trial. The data were subjected to one- and two-way ANOVA to evaluate effects of AC application, heavy metal and pesticide toxicity. All the statistical analyses were done using Minitab software.

III. Results:

The calculated values for ALP along with percent over controls and standard deviations (SD) are given in Table.1 and Fig.1. In the tissues of control and positive control (AC) fish, *Labeo rohita* ALP content was in the order of:

Liver < Kidney < Gill < Intestine

Under exposure of Azoxystrobin, Azoxystrobin+ AC, Copper, Cu+ AC, CS (Combined synergism) and Combined Synergism+ AC for 1 day lethal concentrations, the present content of ALP in the test tissues of *Labeo rohita* was in the order of:

Azoxystrobin, Azoxystrobin+ AC, Copper and Cu+ AC: Liver < Kidney < Gill < Intestine

Combined Synergism: Kidney < Gill < Liver < Intestine

Combined Synergism+ AC: Liver < Kidney < Gill < Intestine

Under exposure of Azoxystrobin, Azoxystrobin+ AC, Copper, Cu+ AC, CS and Combined Synergism+ AC for 1 day sublethal concentrations, the ALP content in the test tissues of *Labeo rohita* was declined in the order of:

Liver < Kidney < Gill < Intestine

Under exposure of Azoxystrobin, Azoxystrobin+ AC, Copper, Cu+ AC, CS and Combined Synergism+ AC for 14 days sublethal concentrations, the present depletion of ALP content in the test tissues of *Labeo rohita* was in the order of:

Liver < Kidney < Gill < Intestine

Under 1 day lethal, sublethal and 14 days sublethal concentrations of selected toxicants, the percentage of ALP increament and depletion were found in the tissues of test fish *Labeo rohita*. Under 1 day lethal exposure the maximum and minimum percentage of augmentation was observed in the intestine (+43.37) and Liver (+1.93) of Combine synergism+ AC and Azoxystrobin+ AC exposures. The abundant and least percentage of depletion was found in the tissues of Liver (-23.99) and (-0.64) of Combined synergism and Combined synergism+ AC. Under 1 day sublethal exposure the maximum and minimum amount of depletion was found in the Gill (-17.62) and Kidney (-0.41) of) of $CuSO_4$+ AC and Azoxystrobin+ AC exposures. After 14 days exposure of sublethal concentration the highest and lowest amount of ALP depletion was observed in the Kidney (-52.29) of Combined synergism and kidney (-18.44) of Az+ AC exposures.

Fig.1. Changes in the ALP content (µg/pi/gram protein/hr) and % change over the control, in different tissue of the freshwater fish, *Labeo rohita* exposed to 1day lethal concentrations of selected toxicants.

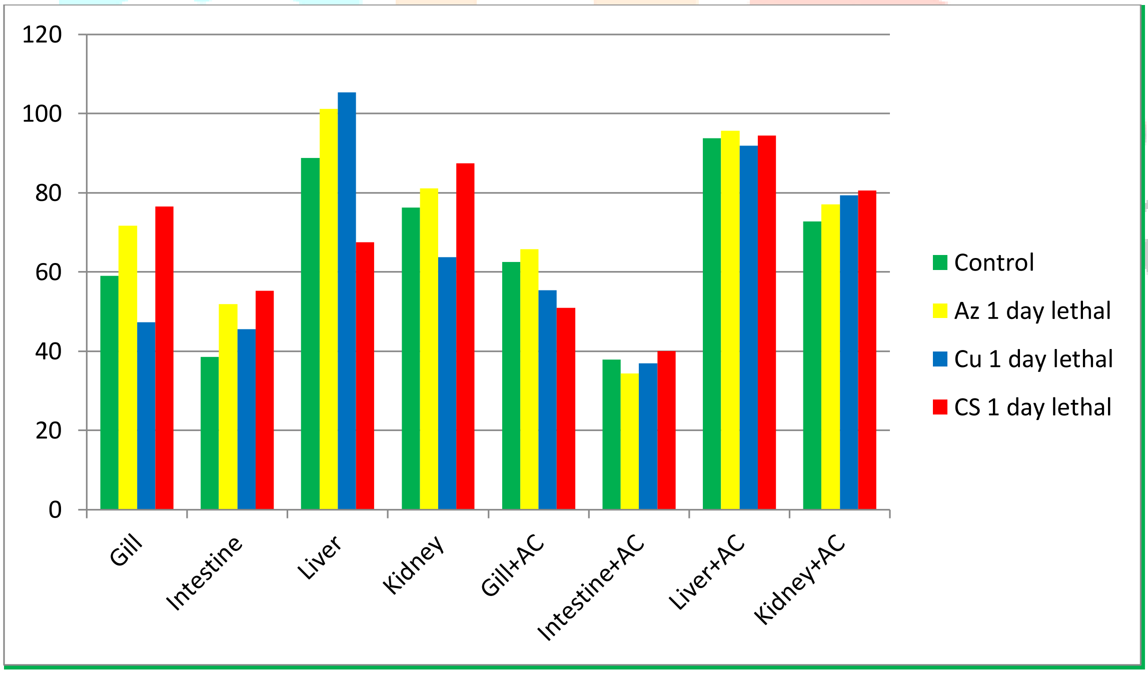


Fig.2. Changes in the ALP content (µg/pi/gram protein/hr) and % change over the control, in different tissue of the freshwater fish, *Labeo rohita* exposed to 1day sublethal concentrations of selected toxicants.

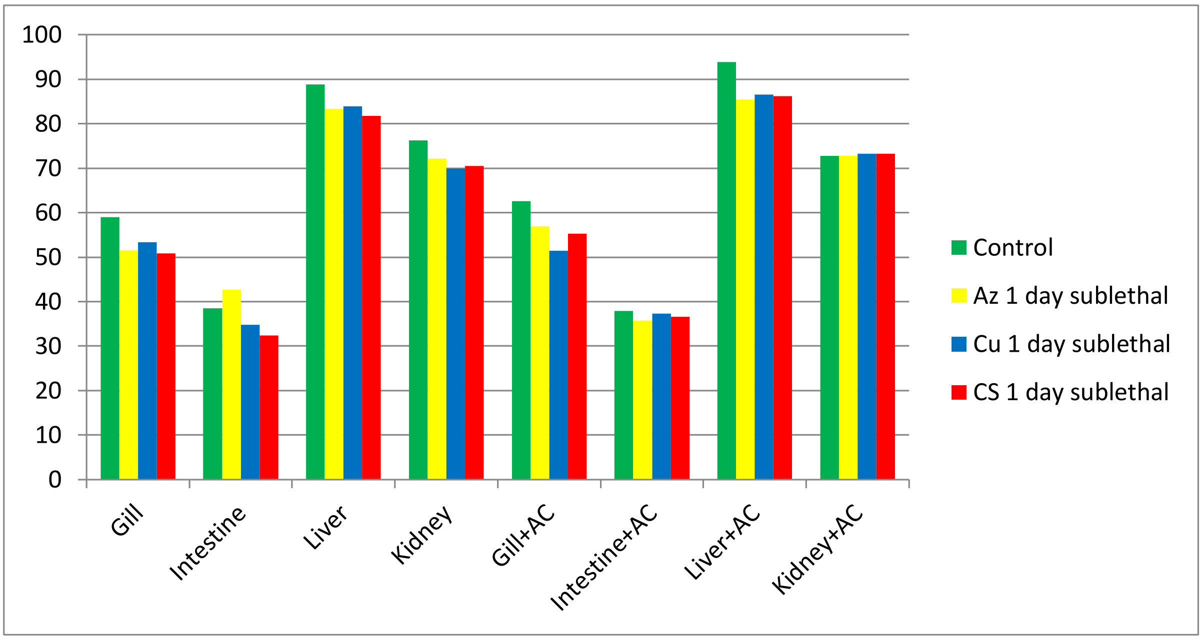
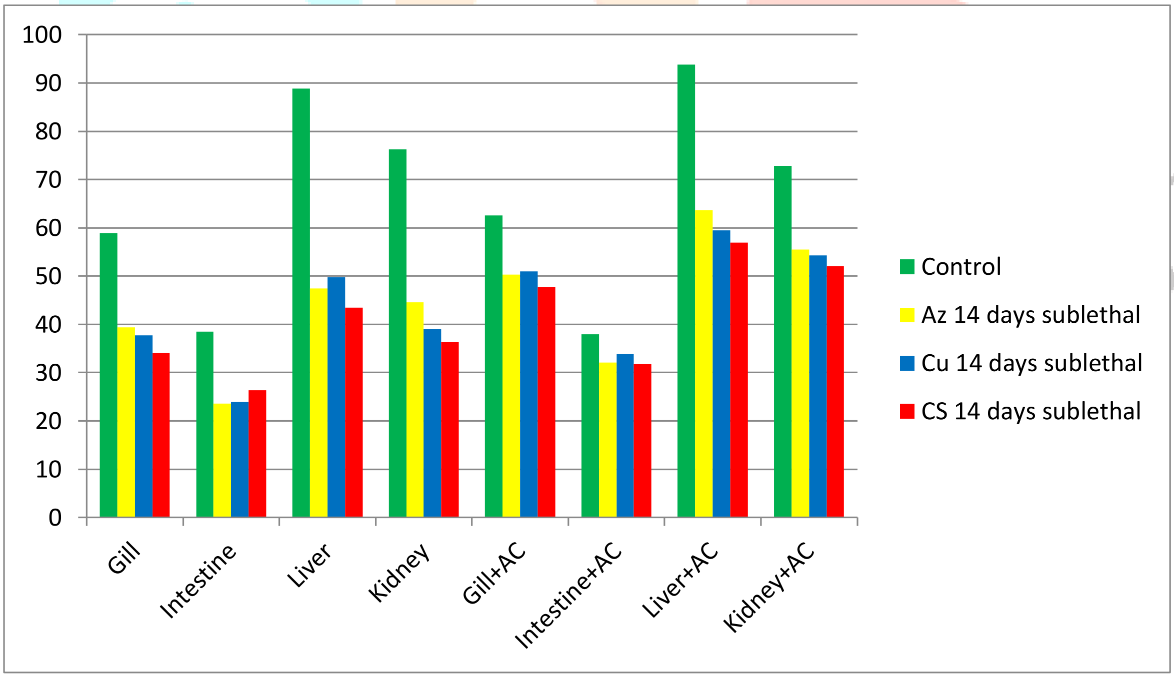


Fig.3. Changes in the ALP content (µg/pi/gram protein/hr) and % change over the control, in different tissue of the freshwater fish, *Labeo rohita* exposed to 14days sublethal concentrations of selected toxicants.



IV. DISCUSSION:

Single-chemical risk assessments are likely to underestimate the impacts of these toxicants on freshwater/ marine water fishes in river systems where mixtures occur. Laetz *et al*., 2009 was stated that several combinations of organophosphates were lethal at concentrations that were sublethal in single-chemical trials. Moreover, mixtures of pesticides that have been commonly reported in aquatic habitats may pose a more important challenge for species recovery than previously anticipated. Azoxystrobin is a strobilurin of growing concern in aquatic environments because it is the most sold fungicide worldwide; however, the information available about its effect of aquatic non-target organisms is scarce. The fungicide azoxystrobin affects mitochondrial respiration and mechanisms controlling cell growth and proliferation in fish and may have negative effects on juvenile Atlantic salmon for 4 days (Olsvik *et al*., 2010). All biochemical variables are significantly affected by AC application, Azoxystrobin, copper exposures and their interaction.

The toxic pollutants may interact with regulators and co-factors and may inhibit enzyme activity, because enzymes are easily altered by even smaller changes, either in internal or external medium. In this study Azoxystrobin, Copper induced alterations in alkaline phosphatase activity individually and combine in the fish *Labeo rohita* after exposures of 1 day lethal, 1 and 14 days sub lethal concentrations. The importance of measuring ALP is to determine the liver dysfunction and the cellular membrane health. Alkaline phosphatase is involved in carbohydrate metabolism, growth and differentiation, protein synthesis, synthesis of certain enzymes, secretion activity, and transport to phosphorylated intermediates across the cell membranes. Elevation in function of ALP activity might be due to an accelerated membrane transport for the function related to anion compounds. Another possibility in activity of ALP may be due to the destruction of the hepatic smooth endoplasmic reticulum membrane.

In the present study elevated levels of ALP was reported in chosen organs of 1 day lethal except gill of copper and liver of CS. Increased activity of ALP on 1 day lethal may be related to hepatic tissue damage and dysfunction due to pesticide toxicity (Fig.1). Under stress conditions of 1 day lethal, the elevated levels of alkaline phosphatases may indicate an increase in the rate of phosphorylation and transport of molecules across the cell membrane, such enhanced phosphatases activity revealed an increase of metabolites transportation through the cellular membrane. Selected toxicants effect was reduced in AC exposed fish and it may enhance the recovery of phosphate level.

After 14 days sublethal exposure experimental fish responded 5.37% TO 51.03% of reduced ALP activity than control and positive control. The observed ALP activity was lower in the experimental fish liver is statistically significant $P < 0.05$ (Fig. 3). A declined activity of alkaline phosphatase in pesticide treated fish may indicate damage in cell organelles like membrane transport system and endoplasmic reticulum. The decreased trend of acid phosphatase activity in fish was mainly due to changes in the mitochondrial membrane function or due to increased glycogenolysis (Parthasarathi and Karuppasamy, 1998). Low amounts of declined ALP found in 1 day sub lethal exposures (Fig.2). While compare to 1 day sublethal to 14 days sublethals decreasing trend was observed and recovery rate noted in AC treated animals.

The decreased alkaline phosphatase activity in gill, intestine, kidney and liver tissue of the test fish exposed to predetermined values of toxicants, indicates that the Azoxystrobin, Copper and Az+Cu act's on plasma membrane and alters the membrane transport. The decreased ALP activity may be due to the destruction of lysosomal membrane (Etim *et al*., 2006). Pooja *et al*., 2020 concluded that chlorantraniliprole interferes with alkaline phosphatase activity in selected test fish *Cirrhinus mrigala* and retards the normal activity. According to my obtained results no one organ may not react in same way to toxicant exposures, it is depending upon the structure, function, composition of organs, amount of enzyme present in organ, availability of pesticides to organs, concentration and duration of exposure periods. The ALP activity exhibits increasing trend in some of them and decreasing trend in others at 1 day lethal. At the end of the 14 days sublethal per cent changes decreased over the controls (Fig.3.). Kuda *et al*., 2002 reported that, the ALP activities in the organs might also be due to leaching from internal organs, apart from stress factor. Furthermore, he revealed that, heavy microbial load in the aquatic system having ALP activity can also lead to accumulation of ALP in different organs. In all treated groups decreasing trend was observed in intestine. Intestinal alkaline phosphatase is controlling gut and systemic inflammation. Gaps in knowledge, e.g. on enzyme isoforms and molecular mechanisms involved in physiological and immunological (inflammatory) responses to biotic and abiotic factors, exist in fish and need to be considered (Jean-Paul Lalles, 2019).

Decreased phosphates activity may be due to alteration in membrane permeability, distribution of normal functioning of cell organelles like lysosomes and mitochondria and different suppressor mechanisms associated with toxicity together resulted in significant changes in the level of enzymes is the tissues examined. The decreased activity of ALP in the tissue is due to the accumulation of metals in fishes (Humstoc *et al*., 2007) which affects the synthesis of enzyme protein directly or indirectly and or increase metabolism due to an increase of toxic substances and the production of toxic metabolic products destructive to enzymes.

V. CONCLUSION:

Enzymes are involved in every physiological process and could serve as a valid biomarker of pollution. The assessment of enzyme activities could be an indicator of pathology. Enzymes are easily altered by even smaller changes, either in internal or external medium. ALP is an intracellular enzyme and used for demonstrating tissue damage in fish as a good diagnostic tool in toxicology studies. The results showed that the pattern of ALP activity was also responds different for different toxicants. Among all treated groups combined synergism exposures showed more effective than individual toxicants. In AC+ toxicant treated groups improved ALP levels noted so AC could reduce the toxic effect of pesticides ad heavy metals. From the present study it may be concluded that long term exposure of organisms to toxicants even at sublethal levels means a continuous health hazard for the fish population. Therefore it is required to monitor the aquatic system and predict the toxic effect of pesticides on fish.

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