ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 9, Issue 4, pp: (1-6), Month: October - December 2021, Available at: www.researchpublish.com

Alterations in the Alkaline Phosphate Activity of Freshwater Fish *Cirrhinus mrigala* Exposed to Methanol

Madhav Bhilave

Division of Fisheries Science

Department of Zoology

Shivaji University, Kolhapur, Maharashtra

mpb_zoo@unishivaji.ac.in +919822874909

Abstract: **Theophrastus Phillipus Auroleus Bombastus von Hohenheim (1493–1541) is credited with the classic toxicology maxim, "*Alle Dinge Sind Gift und nichts ist ohne Gift; allein die Dosis macht, dass ein Ding kein Gift ist.*" which translates as, "All things are poisonous and nothing is without poison; only the dose makes a thing not poisonous." This is often condensed to: "The dose makes the poison". The goal of toxicity assessment is to identify adverse effects of a substance. Adverse effects depend on two main factors: routes of exposure (oral, inhalation, or dermal) and dose (duration and concentration of exposure). To explore dose, substances are tested in both acute and chronic models. Generally, different sets of experiments are conducted to determine whether a substance causes some forms of toxicity. Methanol is an industrial chemical used after 1800s. It is a toxic. This chemical are used to produce biodiesel and also used in ethanol manufacturing industries as a denaturant additive. In the 2010, the American Methanol Institute (AMI) evaluation stated the methanol demand may be reach up to the 882 million gallons per year. In the present study Methanol was selected as Toxicant on fingerlings of freshwater fish *Cirrhinus mrigala* to understand the alterations in Alkaline Phosphates after acute exposure. It was observed that Alkaline Phosphates decreased as the dose component was increased.**

Keywords: **Toxicant, Methanol, Alkaline phosphates activity, Freshwater fish.**

1. INTRODUCTION

Aquatic toxicology is the part of science which incorporates investigation of the impacts created by substance on sea-going life forms at different levels of association in biological systems. Sea-going toxicology is an interdisciplinary field which includes oceanic biology, toxicology and amphibian science. The field of sea-going toxicological investigation incorporates freshwater, marine water and residue conditions. Harmfulness test incorporates intense poisonous quality tests (24– 96 hours) and perpetual lethality test (at least 7 days). The Acute poisonous quality test incorporates antagonistic impacts of compound to a living being amid here and now presentation and ceaseless danger has a capacity of a substance to create destructive impacts over a long haul period. Numerous methodologies have been concentrated to assess the intense and endless toxicological impacts of contaminations in crisp water biological communities. Lethality tests not just exhibits that a compound is protected or not, but rather likewise portrays the impacts, a synthetic can create or not. The motivation behind these tests is to dole out far reaching data in hazard assessment.

Sea-going contamination with concoction contaminants is a standout amongst the most basic ecological issues of the century. Streams, lakes and other sea-going bodies are dirtied with mechanical waste water, squanders from family unit exercises and farming run offs. Unregulated development of urban regions, without infrastructural administrations for

legitimate accumulation, transportation, treatment and transfer of household squanders prompts expanded sea-going contamination. Contaminations and their synthetic constancy from various mechanical territories in the earth and freshwater biological systems are looked with spatially or transiently alarmingly elevated amounts of xenobiotic synthetic compounds (Brack *et al*., 2002; Diez *et al*., 2002). A portion of these synthetics are biodegradable while others are non-biodegradable; they remain hazardously for quite a while interim.

Methanol, the substance chose for present examination, is a dismal, fluid and combustible compound with the equation CH_3OH utilized as a mechanical concoction after 1800s. It is a lethal compound utilized as a dissolvable, radiator fluid and fuel. Methanol is incorporated from the damaging refining of wood and furthermore from carbon monoxide and hydrogen. Methanol is harmful, when 10 ml of the substance is devoured incidentally by drinking, it cause visual deficiency and 100 ml results in the passing of a person. These synthetic concoctions are utilized to create biodiesel, and utilized in ethanol fabricating ventures as a denaturant added substance.

Fishes are considered as a vital bio-marker in amphibian environments. Fishes are all of a sudden influenced by the smallest change in water contamination since it is in direct contact with the water. Fish liver and kidney are associated with detoxification, osmoregulation, biotransformation and discharge of xenobiotics (Vesey, 2010). Fishes are extremely delicate to defilement of water, in this manner; toxins may altogether adjust some biochemical and physiological systems when they go into the organs of fishes (Banaee *et al*., 2011, Shrivastava *et al*., 2004).

The Indian Major Carps are delicacy when contrasted with other freshwater angle species. *Cirrhinus mrigala*, the chose angle for present experimentation, is the benthopelagic, potamodromous, microscopic fish feeder and has a quick development rate. It flourishes in quick streaming streams and waterways. Bringing forth happens in the negligible regions of the water bodies with a profundity of 50 to100 centimeters over a sand or earth substrate amid rearing season i.e. storm season. The Cirrhinus mrigala neglects to breed normally in appropriated water bodies like lakes; henceforth they are subjected to instigate rearing (Rema Devi *et al*., 2011). *Cirrhinus mrigala* is a prominent nourishment angle. It is generally cultivated as a segment of a polyculture framework.

2. MATERIALS AND METHODS

Acute toxicity:

Acute toxicity describes the adverse effects of a substance that result either from a single exposure or from multiple exposures in a short period of time (usually less than 24 hours). To be described as acute toxicity, the adverse effects should occur within 14 days of the administration of the substance. Acute toxicity is distinguished from chronic toxicity, which describes the adverse health effects from repeated exposures, often at lower levels, to a substance over a longer time period (months or years). Most acute toxicity data comes from animal testing (here fish as animal model) or, more recently, *in vitro* testing methods and inference from data on similar substances. The acute toxicity of a chemical refers to the effects from a single dose or repeated exposure over a short time. Toxicant with a high acute toxicity leads to death, even if a small amount is absorbed. Acute exposures may also be considered as acute poisoning, acute oral or acute dermal.

LC_{50} or Lethal Concentration 50:

The LC_{50} concentration means the concentration of toxicant which is lethal to 50% of a population of test animals and is usually determined for a specific exposure period. The length of exposure is an important factor because; shorter exposure period needs higher toxicant concentrations to produce toxic effects. LC_{50} values for toxicant determined in parts per billion (ppb) or parts per million (ppm). LC_{50} value for fish or other aquatic animals depend on the concentration of toxicant in water for exposure periods of 24 to 96 hours.

Alkaline Phosphates:

Alkaline phosphates (E. C. 3. 1. 3. 1, Orthophosphoric Monoester phosphohydrolase) is a transmembrane glycoprotein (Kaplan 1972). It is commonly dispersed in living prokaryotic and eukaryotic organisms. Alkaline phosphates are a diametric enzyme commonly confined to the plasma membrane. It hydrolyzes the phosphate monoesters at alkaline PH (McComb 2013). In mammals, alkaline phosphates are a metalloenzyme containing two molecules of zinc and one molecule of magnesium present at the active site. The metal ions in the enzyme maintain the configuration of the enzyme.

This metal ion also contributes significantly to the structure of alkaline phosphates monomer and indirectly regulates subunit interactions (Plocke et al., 1965). Prokaryotic and eukaryotic organisms contains various is form of alkaline phosphates. These isoforms are based on the tissue where alkaline phosphates are expressed. They are placental, liver/bone/kidney and germ cell alkaline phosphates (Griffiths and Black 1987).

In human, placental alkaline phosphates are located in chromosome 2 and have 80% homology. It is heat stable and present in placenta and little amounts is detected in sera (Neale et al., 1965). Intestinal alkaline phosphates are partially heat stable. It differs from other alkaline phosphates due to absence of sialic acid on the carbohydrate side chain. The fetal and adult intestinal alkaline phosphates can be isolated. The fetal intestinal alkaline phosphates are salivated (Bitar and Reinhold 1972). A germ cell alkaline phosphate is heat stable and is present in germ cells as well as in neoplastic tissues. It is also presents in the cell membrane of immature germ cell, testis cells and lesser amounts in the placenta. It is attached to the cell membrane by the phosphatidyl-inositol-glycan anchor (Ginsburg et al., 1990). Liver/bone/kidney alkaline phosphate is a heat liable and tissue non-specific enzyme. It is present in many tissues throughout the body and is at higher level in skeletal tissue, kidney and liver. All these tissue enzymes are encoded a single gene locus at chromosome 1. Even though alkaline phosphates are present in the various tissues, they have different physiological properties, but all catalyze the same type of reaction (Weiss et al., 1988).

Alkaline phosphates are a transmembrane glycoprotein of the plasma membrane. It is involved in active transport mechanism, bone calcification, metabolism of carbohydrate, nucleotides and phospholipids (McComb 2013). The plasma membrane acts as the first barrier to toxicants. Any damage to plasma membrane results in alternations in alkaline phosphates activity.

Estimation of Alkaline Phosphates activity

The muscle, intestine and liver tissues were pooled from sacrificed fishes and acid phosphates and alkaline phosphates activity was estimated by Linhardt and Walter (1965) method using p-nitro phenyl phosphate as the substrate. Homogenate of each tissue was prepared in 0.9% chilled saline solution and centrifuged at 3000 rpm for 10 minutes. For the assay triplet set of test tubes were prepared for the each tissue. Thereafter, 0.2 ml supernatant was added in each test tube. Then 0.8 ml 0.05M sodium citrate buffer containing 5 X 10^{-3}M p-nitro phenyl phosphate pH 4.8 was added for acid phosphates and 0.8 ml 0.05M sodium citrate buffer containing 5 X 10^{-3}M p-nitro phenyl phosphate pH 7.6 was added for alkaline phosphates. The blank was prepared by adding 0.2 ml distilled water and 1 ml substrate buffer and all tubes were incubated at 37^0C for 30 min. Then 4 ml of 0.1 N NaOH was added for inhibition of reaction. The absorbance value was measured at 400 nm by spectrophotometrically using blank. The lysosomal acid phosphates and alkaline phosphates activity in term of µmols of p-nitro phenol /mg protein was calculated by the formula:

Phosphates activity = Optical Density X 2.76 X1000 / amount of protein/mg tissue.

3. RESULT

The result of the alkaline phosphates activity in term of p-nitro phenyl phosphate/mg tissue protein after acute exposure (96 hours) of Methanol in various tissue of freshwater fish *Cirrhinus mrigala* viz. muscle, intestine and liver in the control group, LC_0 concentration group and LC_{50} concentration group are shown in Table No.1 and Graph No.1. The muscle tissue of the control group fish exhibited 3.69 ± 0.17 µm of p-nitro phenol phosphate/mg protein in tissue. While in the LC_0 concentration group fish showed 1.92 ± 0.02 µm of p-nitro phenol phosphate/mg protein in tissue of muscle and in the LC_{50} concentration group was 1.18 ± 0.1 µm of p-nitro phenol phosphate/mg protein in tissue of muscle. In the intestine of the control group, the acid phosphates activity was 13.54 ± 0.72 µm of p-nitro phenol phosphate/mg protein in tissue. However, in LC_0 concentration group fish intestine exhibited 6.67 ± 0.17 µm of p-nitro phenol phosphate/mg protein in tissue and in the LC_{50} concentration group fish showed 3.69 ± 0.26 µm of p-nitro phenol phosphate/mg protein in tissue of intestine.

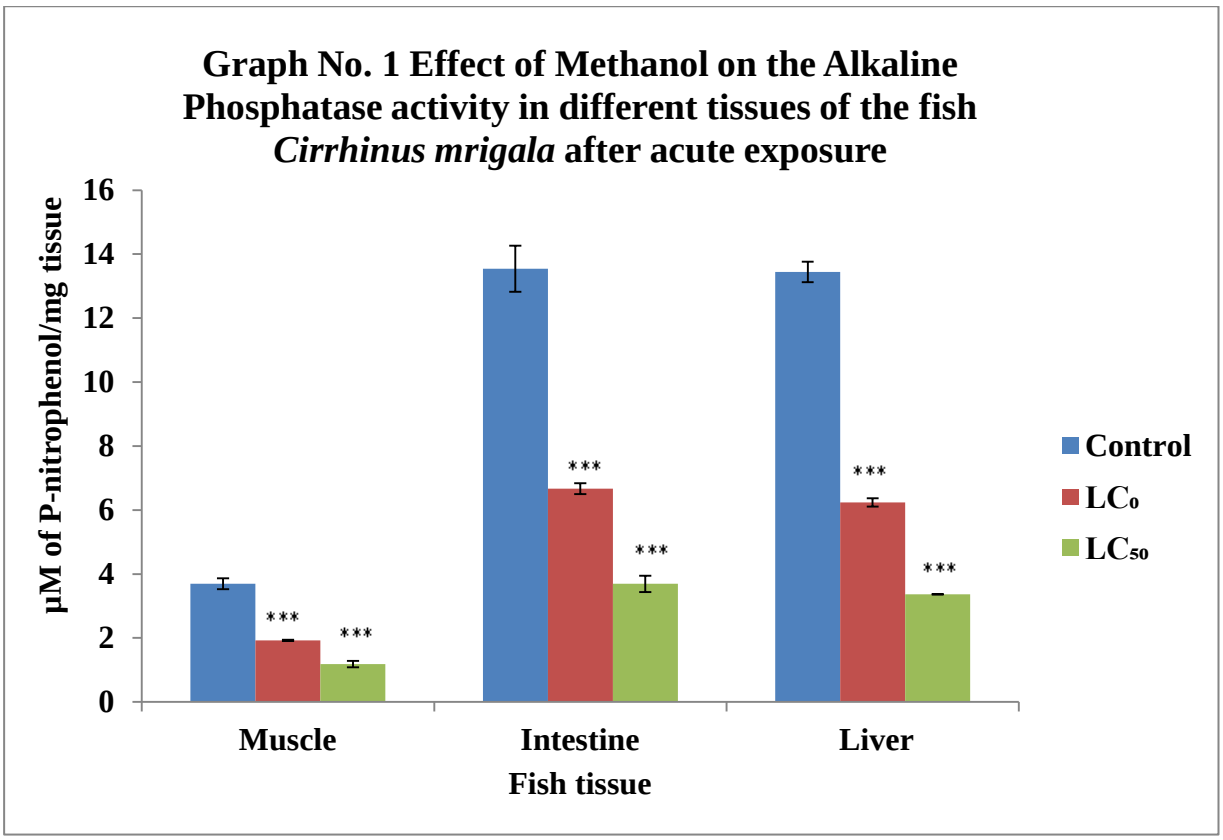
In the control group fishes, 13.44 ± 0.32 µm of p-nitro phenol phosphate/mg protein in tissue of the liver, while in the LC_0 concentration group fishes exhibited 6.24 ± 0.13 µm of p-nitro phenol phosphate/mg protein in tissue of liver and in the LC_{50} concentration group fishes it was 3.36 ± 0.01 µm of p-nitro phenol phosphate/mg protein in tissue of liver. The alkaline phosphates activity in the muscle, intestine and liver tissues after exposure of 96 hours of Methanol at LC_0 and LC_{50} concentration was decreased as compared to the control group. The difference was highly significant at $p<0.001$.

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 9, Issue 4, pp: (1-6), Month: October - December 2021, Available at: www.researchpublish.com

Table No. 1 Effect of Methanol on the Alkaline Phosphates activity in different tissues of the fish *Cirrhinus mrigala* after acute exposure

Groups	µm of p-nitro phenol phosphate/mg protein in tissue		
	Muscle	Intestine	Liver
Control Group	3.69±0.17	13.54±0.72	13.44±0.32
LC_0	1.92±0.02***	6.67±0.17***	6.24±0.13***
LC_{50}	1.18±0.1***	3.69±0.26***	3.36±0.01***

(Values expressed is mean of (n=5); ±SD) *=P<0.05; **=P<0.01; ***=P<0.001; NS= > 0.05



(Values expressed as Arithmetic Mean of (n=5); ±SD), *** indicate P<0.001

4. DISCUSSION

Water is one of the most important compounds in the ecosystem. All living organisms on the earth need water for their survival and growth. Due to increased human population, industrialization, use of fertilizers in the agriculture and man-made activity it is highly polluted with different harmful contaminants. Physicochemical characteristics of the water play an important role in the effects of toxicants on the growth and survival of aquatic organisms. The parameters like pH, temperature, hardness, salinity and dissolve oxygen in water usually affects on aquatic organisms. The level of toxicant in fish tissues is influenced by many factors such as biotic, abiotic and environmental which influence on fish growth and mortality rate. The fish species, age of fish, habitat, concentration of toxicant, exposure period, temperature, salinity of water, pH in water, dissolved oxygen in water and other physiological conditions modify toxicity to the fish.

In the present study, the 96 hr LC_{50} value of Methanol to *Cirrhinus mrigala* was observed to be 12,250 mg/L with 95% confidence limit ranging in between 14,230 to 11,270 mg/L. Fish exposed to methanol showed excess mucous secretion from skin. Powers (1917) reported that 250 mg/L methanol kills goldfish *Carassius auratus* in distilled water within11-15 hrs. Liebmann (1960) listed the critical lethal concentration of methanol for narcosis as 31,000 mg/L. Dawson *et al*., (1970) cited threshold concentration of methanol for fish is 240 mg/L. Benville and Mauck (1971) found that the 96 hrs TL_{50} of Methanol for rainbow trout was 15,000 mg/L.

Methanol is converted into the formaldehyde through alcohol dehydrogenate and then formaldehyde is converted into formic acid or formate with the help of aldehyde dehydrogenate. This formate is toxic because it inhibits mitochondrial cytochrome c oxidize, causing hypoxia at the cellular level, and metabolic acidosis, resulting different physiological problems or metabolic disturbances depending upon its concentration Liesivuori and Savolainen (1991). Tephly (1991) observed hyperactivity and convulsion of fish exposed to methanol. Helmstetter *et al*., (1996) reported the sluggish movements with extended siphon and slow reflexes in mussel caused by methanol administration. A scant literature is available on chronic effects of sub lethal concentrations of methanol to fish and aquatic ecosystem.

It is well-known fact that, alkaline phosphates are actively involved in membrane transport and transphosphorylation reactions in cell (McComb*et al*., 2013). The resultant decrease is attributed to damage of plasma membrane after exposure to Methanol (Radhakrishnan and Jasmine 2010). Parthasarathi and Karuppasamy (1998) observed that, decrease in alkaline phosphates activity in muscle cells is due to uncoupling of phosphorylation, increase glycogenolysis and altered functions of mitochondria. Priyatha and Chitra (2019) observed that, exposure of toxicant acid orange 7 inhibits the alkaline phosphates activity and altered membrane transport. This leads to breakdown of glycogen as a result of dye intoxication.

5. SUMMARY AND CONCLUSION

The alkaline phosphates activity in various organs viz. muscle, intestine and liver of the fish *Cirrhinus mrigala* after acute exposure (96 hours) in LC_0 concentration groups and LC_{50} concentration groups was significantly decreased as compared to control group. The inhibition of alkaline phosphates activity is due to uncoupling of phosphorylation, increase glycogenolysis, altered functions of mitochondria and elevated levels of Methanol in tissues.

Present studies reveals that the selected toxicant Methanol at various concentrations exhibited mortality in freshwater fish *Cirrhinus mrigala*, and based on rate of mortality, the values of LC_0 and LC_{50} were calculated in terms of observed and calculated. Exposure of experimental fish to acute concentrations; leads to changes in metabolic and enzymatic activities. Variations in Alkaline Phosphates profile show the vulnerability and toxic intensity of Methanol on various tissues. Further studies are required to understand the severity of Methanol at cellular and molecular levels and to understand the impact of depuration behavior of exposed fishes after time interval.

REFERENCES

[1] Banaee, M., and Ahmadi, K. 2011. “Sub-lethal toxicity impacts of endosulfan on some biochemical parameters of the freshwater crayfish (Astacus leptodactylus).” *Research Journal of Environmental Sciences* 5 (11):827-835

[2] Benville P E and Mauck WL. 1971. “Toxicology of Pesticides in Aquatic Organisms of the

[3] Great Lakes.” *Annual Progress Report of the Fish Pesticide Research Laboratory, US Bur SportFish Wildly, Columbia, MO*

[4] Bitar, K., & Reinhold, J. G. (1972). Phytase and alkaline phosphates activities in intestinal mucosa of rat, chicken, calf, and man. *Biochemical et Biophysica Acta (BBA)-Enzymology*, *268*(2), 442-452

[5] Brack C.L. 2002. “Pollution mitigation and carbon sequestration by an urban forest.”*Environmental Pollution*116: 195–200

[6] Dawson G W, Shuckron A J and Swift W H. 1970. “Control of spillage of hazardous polluting substances.” US Dept of Interior, Fed Water Qual Adm, Rept No. 15090 FQZ 10/70

[7] Diez, Thomas 2002 The European Union and the Cyprus Conflict: Modern Conflict, Postmodern Union (Manchester: Manchester University Press), 139-62

[8] Geissmann, F., Manz, M. G., Jung, S., Sieweke, M. H., Merad, M., & Ley, K. (2010). Development of monocytes, macrophages, and dendrite cells. *Science*, *327*(5966), 656-661

[9] Griffiths, J., & Black, J. (1987). Separation and identification of alkaline phosphates isoenzymes and isoforms in serum of healthy persons by isoelectric focusing. *Clinical chemistry*, *33*(12), 2171-2177

[10] Helmstetter, A., A. P. Gamerdinger, and R. J. Pruell. 1996. Acute Toxicity of Methanol to *Mytilus edulis*. Bull. Environ. Contam.Toxicol. 5:675–681

[11] Kaplan, M. M. (1972). Alkaline phosphates. *Gastroenterology*, *62*(3), 452-468

[12] Linhardt K, Walter K. (1965). Phosphates (phosphomonoesterases). In Methods of enzymatic analysis. (pp. 779-787). Academic Press

[13] Liebmann H 1960 Hanbuch derFrischwasser-und-Abwasser biologic II. R Oldenburg, Munich, 679-693.

[14] Liesivuori J and Savolainen H 1991. "Methanol and formic acid toxicity: biochemical mechanisms." *Pharmacol. Toxicol* 69 (3): 157–63

[15] McComb, R. B., Bowers Jr, G. N., & Posen, S. (2013). Alkaline phosphates. Springer Science & Business Media

[16] Neale, F. C., Club, J. S., Hotchkis, D., & Posen, S. (1965). Heat stability of human placental alkaline phosphates. *Journal of clinical pathology*, *18*(3), 359-363

[17] Plocke, D. J., Leviathan, C., & Vallee, B. L. (1962). Alkaline phosphates of *Escherichia coli*: a zinc metalloenzyme. *Biochemistry*, *1*(3), 373-378

[18] Powers E B 1917. "The Goldfish (*Carassius carassius*) As a Test Animal in Study of Toxicity."*Illinois Biol Mono, Urbana, IL*. 4:127-193

[19] Remya V 2010. "Biochemical Effects of different Phenol compounds on *O. mossambicu*." Ph.*D. Thesis. Cochin University of Science and Technology, Cochin.*

[20] Shrivastava and S. Sing 2004. "Changes in Protein Content in the Muscle of *Heteropneustes fossilis* exposed to Carbaryl." *Journal of Ecotoxicology and Environmental Monitoring* 14: 119-122

[21] Tephly TR 1991 "The toxicity of methanol."*Life Sci*. 48(11):1031-41

[22] Vesey DA 2010. "Transport pathways for cadmium in the intestine and kidney proximal tubule: focus on the interaction with essential metals."*Epub* 198(1):13-9

[23] Weiss, M. J., Ray, K., Henthorn, P. S., Lamb, B., Kadesch, T., & Harris, H. (1988). Structure of the human liver/bone/kidney alkaline phosphates gene. *Journal of Biological Chemistry*, *263*(24), 12002-12010