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Enzymological Effects of Lead Acetate Exposure in Various Organs of Oreochromis mossambicus (Peters 1852)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The various enzymological effects of heavy metal Lead acetate $(Pb(C_2H_3O_2)_2)$ exposed to fingerlings of *Oreochromis mossambicus* was analyzed in this study. The 30 days old fingerlings of *O. mossambicus* were exposed to sub lethal dose 3.80ppm of 96 hrs. LC_{50} of $Pb(C_2H_3O_2)_2$ for 21 and 28 days, various organs (liver, gill, kidney and muscle tissue) were removed from control and treated fishes and were used for various enzymological effect: Succinate dehydrogenase (SDH), Lactate dehydrogenase (LDH), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Acid phosphatase (ACP) and Alkaline phosphatase ALP). The observed data were subjected into various statistical analysis by using IBM-SPSS version 26. Among the two different exposure periods 21 and 28 days which compared with control fingerlings organs, the various enzymological

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effects were drastically suffered (Treated fish enzymological levels were inversely proportionate towards the control groups of fish species) by 28 days of exposure periods. Pb(C₂H₃O₂)₂ exposed to fingerlings which tested on various organs at 28 days interval periods for assessing of SDH, LDH, AST, ALT, ACP and ALP of various organs of treated fish: Liver: 12.02, 55.75, 58.24, 125.27,12.26, 11.82 μ moles of phenol liberated/ mg of protein; Gill: 10.80, 33.75, 28.73, 28.11, 7.80, 8.63 μ moles of phenol liberated/ mg of protein; Kidney: 12.18, 46.33, 44.78, 75.40, 6.27, 10.38 μ moles of phenol liberated/ mg of protein and Muscle: 11.72, 32.44, 32.76, 44.20, 3.72, 7.20 μ moles of phenol liberated/ mg of protein were recorded on tissue of treated fish. The enzymological effect of Pb(C₂H₃O₂)₂ exposed towards *O. mossambicus* which observed various health defects on organism exposed to a particular toxicant. It may give sufficient information about heavy metal contamination on aquatic organism and would help us to propose policies to protect the ecosystem.

Keywords: Oreochromis mossambicus; Pb(C₂H₃O₂)₂; enzymological effects; various organs; metal toxicant.

1. INTRODUCTION

The fish Tilapia is one of the prime marketable fish and it alone contributes above 85% production around the world (Hasan et al. 2021). Tilapia is a fast-growing aquatic species and it can be marketable within few months as well as highly profitable, tastiest and nutritional products in many of the poor nations (Afrose et al. 2021). It is one of the efficient bio-indicator for aquatic environment as well as it is recently used as model fish to observe the toxicity of aquatic environment (Moyo et al. 2021). Most of the industrial effluents are highly toxic nature which are greatly exposed through anthropogenic activities as well as finally end with aquatic ecosystems (Naylor et al. 2021). The continuous discharging of toxicants highly polluted in aguatic environment as the result countless of defects in aquatic species, they are supporting destructive effects on aquatic species which promote various health defects: physiological and anatomical metabolic disorder defect, and cellular malfunctions of living things (Naylor et al. 2019). The grater contamination of heavy metal highly causing on aquatic resources which drastically exploit aquatic ecosystem (Abu-Elala et al. The major way of heavy metal 2023). accumulation in aquatic ecosystem through water and food which leads less active functioning of various physical and anatomical activities of aquatic organisms as the results damages occurred in gills, skin, liver, kidney, digestive passages, respiratory and circulatory organs, etc., (Flora 2011). Metal mining, smelting, refining, and processing, along with fuel combustion, and waste incineration activities release significant amounts of Pb(C₂H₃O₂)₂ into freshwater habitats through atmospheric deposition and in liquid effluents and leachates

(Ju-Wook et al. 2019). Limited study only has assessed fish toxicology and enzymological effects in fish species under laboratory condition and the examination have done in aquatic organisms by the exposure of $Pb(C_2H_3O_2)_2$. Hence, the present study focusses on heavy metal $Pb(C_2H_3O_2)_2$ induced enzymological changes in the liver, gill, kidney and muscle tissue of *O. mossambicus*.

2. MATERIALS AND METHODS

2.1 Experimental Organism

The aquatic fauna, O. mossambicus were cautiously obtained (Diseases free. even sized, 15day old fingerlings) from east coastal Poompuhar Village, zone Mayiladuthurai District, Tamilnadu, India. The obtained fingerlings (around 100 nos. for each container) gently transported air-filled were to polythene container CI free H₂O. This mode of transit proved successful, since there was no mortality in all consignments throughout the course of this study. The obtained fingerlings were maintained separately under laboratory condition. The fingerlings were transferred for acclimatization in large rectangular tanks (150X100X75cm) of 1000 lit. capacity for a fortnight before they were used for the experiment. The tanks were maintained neat and clean from the fungal infection by washing with KMnO₄ solution. The fingerlings were disinfected with 0.1% KMnO₄ solution and were maintained for three weeks in well-aerated CI free H₂O. to CI free H₂O and fully aerated environment in the rectangular tanks (100X100X100cm) of 1000 lit. capacity. The fingerlings maintained in 12 hrs. light and 12 hrs. dark, pH range between 6.90 to 7.10 and

temperature ranging between 18 to 22 °C for 15 days as well as maintained all physiochemical parameters.

2.2 Experimental Design

The fingerlings were separated into two equal batch each comprising of 20 fishes (30 days old), and the size selected for the experiments were 80-100mm length and 5-10g of weight. Each batch was maintained in separate transparent glass container. The first batch was maintained as control (Without treatment). The second batch was exposed to a sub-lethal concentration of $Pb(C_2H_3O_2)_2$ 3.80ppm. Both the batches of fingerlings were reared for 30 days. The toxicant was renewed every 24hrs, exposure tenure. At end of the treatment, (After the exposure period 21 and 28 days) fingerlings were separated as control and treatment batches. the fingerlings various organs (liver, gill, muscle and kidney tissue) were subjected to assess the estimation of various enzymological study SDH, LDH, AST, ALT, ACP and ALP.

2.3 Estimation of Biochemical Studies

Samples preparation: The treated fish, O. mossambicus liver, gill, muscle and kidney organs were separated from the animal, 5 percent homogenate was prepared in 0.25m C₁₂H₂₂O₁₁ solution and centrifuged at 2500 rpm for 15 minutes to remove cell debris. The supernatant was used for the enzyme assay. Succinate dehydrogenase (SDH): The reaction mixture consisting of 1.0ml of Na⁻K⁻phosphate buffer (0.1M pH 7.4), 0.5 ml of sodium succinate (0.1M pH 7.4) and 0.5ml of 0.5 percent INT (2--3-(P-nitrophenyl) (P-iodophyenyl) -5-phenyl tetrazolium chloride) (Nachales et al. 1960). Lactate dehydrogenase (LDH): In a 10 ml clean test tube the following reaction mixture consisting of 1.0ml of 0.1 M Na⁻ K⁻ phosphate buffer, 0.5ml of 0.1M lithium lactate. 0.5 ml of 5 percent INT in water was added. All the above dehydrogenase reactions were initiated by the addition of 1.0 ml tissue homogenates. The samples were incepted at 37°C for 1 hour and the reactions were stopped by the addition of 6.0ml of CH₃COOH. The formazan formed was extracted with 6.0ml of C₆H₅CH₃ by keeping the tubes overnight in a refrigerator at 5°C. The color was read at 495 nm double beam spectrophotometer. in The dehydrogenase activity was expressed in µ moles formazan formed / mg protein / hour (Govindappa et al. 1965).

Aspartate amino transferase (AST): The amount of oxaloacetate was measured by converting it into pyruvate treating with aniline citrate and then made to react with 2, 4 dinitro phenyl hydrazine. The absorption of brown colour was read at 520 nm. lanine aminotransferase (ALT): The amount of pyruvate formed was measured by treating the pyruvate with 2, 4 dinitro phenyl hydrazine. The absorption of brown colour was read at 520nm (Tennis Wood 1976). Acid phosphatase (ACP) and Alkaline phosphatase (ALP): The organs were homogenized in glass homogenizer using 10ml distilled water. This content was centrifuged at 3000 rpm for 10 minutes. In a clean test tube 0.5 ml of supernatant was taken and 0.5ml of the substrate solution (p-nitrophenyl phosphate) and 0.5 ml of 0.1 N citrate buffers for Acid phosphatase and glycine buffer for Alkaline phosphatase were added. The test tube with the above solution was kept in a water bath maintained at 37°C for 30 minutes. After completion of 30 minutes, the reaction was arrested in the extracts by adding 3.8 ml of 0.1N sodium hydroxide. The colour formed at the end was read at 415 nm in UV spectrophotometer. Phenol was used to construct the standard graph. The values are expressed in µ moles of phenol liberated / min / 100 mg of protein (Masola et al. 2008).

2.4 Statistical Analysis

The observed data were subjected into various statistical analysis (Mean and Standard Error) by using IBM-SPSS version 26. The percentage of bio-activities five batches (Control and treatment) were calculated by replication of six times and the statistically significance at p<0.05 and subjected into student 'T' test analysis.

3. RESULTS AND DISCUSSION

The selected concentration of heavy metal fingerlings $Pb(C_2H_3O_2)_2$ exposed to of Oreochromis mossambicus which tested on various organs such as Liver, Gill, Kidney and Muscle in various interval periods (21 and 28 days) for assessing treatment and control against enzymological (SDH, LDH, AST, ALT, ACP and ALP) studies. Among the two different exposure periods 21 and 28 days which compared with control fingerlings organs, the various enzymological effect were drastically suffered by 28 days of exposure periods. Pb(C₂H₃O₂)₂ exposed to fingerlings which tested on various organs at 28 days interval periods for assessing of SDH, LDH, AST, ALT, ACP and ALP of

Part used	Parameters	Control	Exposure 21 days	% change	Exposure 28 days	% change
Liver	SDH	51.37±2.49 ^d	16.90±0.52 ^d	-30.47	12.02±0.33°	-62.28
	LDH	29.12±2.66 ^d	49.28±1.69 ^d	52.37	55.75±2.76 ^d	70.44
	ALT	44.64±0.22 ^d	57.36±5.49 ^d	14.28	58.24±4.35 ^d	22.46
	AST	62.24±1.76 ^d	118.38±2.75 ^d	74.80	125.27±1.22 ^d	28.33
	ACP	3.37±0.22 ^d	10.28±0.33 ^d	47.51	12.26±0.64 ^d	55.14
	ALP	5.22±0.78 ^d	10.84±0.49 ^d	42.27	11.82±0.22 ^d	53.64
Gill	SDH	38.22±1.38 ^b	15.67±0.95°	-32.77	10.80±0.48 ^a	-56.30
	LDH	18.33±1.28 ^b	30.42±1.76 ^b	40.46	33.75±2.86 ^b	31.54
	ALT	20.46±0.12 ^a	25.68±0.37ª	16.97	28.73±1.64 ^a	19.37
	AST	14.31±0.67 ^a	25.38±1.48ª	36.21	28.11±1.76 ^a	40.86
	ACP	2.78±0.56°	6.18±0.51°	44.60	7.80±0.46°	41.24
	ALP	3.43±0.76 ^b	7.60±0.76 ^b	24.89	8.63±0.44 ^b	62.43
Kidney	SDH	42.37±1.46°	15.44±0.46 ^b	-26.37	12.18±0.38 ^d	-46.72
	LDH	26.08±0.73°	42.38±2.76°	55.88	46.33±1.54°	15.20
	ALT	30.43±0.42°	42.47±2.00°	15.18	44.78±2.33°	24.75
	AST	42.77±1.38°	65.72±0.22°	28.27	75.40±2.57°	52.43
	ACP	2.74±0.27 ^b	5.18 ±0.45 ^b	24.38	6.27 ±0.28 ^b	80.66
	ALP	4.76±0.26°	9.28±0.76°	28.00	10.38±0.42°	54.40
Muscle	SDH	33.25±0.28 ^a	14.38±0.46ª	-77.13	11.72±0.55 ^b	-61.76
	LDH	16.75±0.42 ^a	28.76±1.81ª	42.76	32.44±1.64 ^a	60.77
	ALT	22.58±0.46 ^b	29.47±1.37 ^b	15.13	32.76±1.99 ^b	18.35
	AST	30.24±0.44 ^b	40.73±1.14 ^b	21.38	44.20±1.28 ^b	30.85
	ACP	1.76±0.82 ^a	3.27 ±0.43ª	45.67	3.72 ±0.38 ^a	63.80
	ALP	2.68±0.55 ^a	6.55±0.39 ^a	37.50	7.20±0.32ª	34.27

Table 1. Enzymological effects of Pb(C₂H₃O₂)₂ in various organs of O. mossambicus

The data were statistically evaluated into Mean ± Standard Error

The percentage of bio-activities were calculated by replication of six times

The Statistically significance at p<0.05 and subjected into student 'T' test.

SDH: Succinate dehydrogenase (µmole formazone formed/mg of protein/hr)

LDH: Lactate dehydrogenase (µmole formazone formed/mg of protein/hr)

AST: Aspartate aminotransferase (µmoles of pyruvate formed / mg of protein/hr)

ALT: Alanine aminotransferase (µmoles of pyruvate formed / mg of protein/hr)

ACP: Acid phosphatase (µ moles of phenol liberated/ mg of protein)

ALP: Alkaline phosphatase (µ moles of phenol liberated/ mg of protein)

various organs of treated fish: Liver: 12.02. 55.75, 58.24, 125.27.12.26, 11.82 u moles of phenol liberated/ mg of protein; Gill: 10.80, 33.75, 28.73, 28.11, 7.80, 8.63 µ moles of phenol liberated/ mg of protein; Kidney: 12.18, 46.33, 44.78, 75.40, 6.27, 10.38 µ moles of phenol liberated/ mg of protein and Muscle: 11.72, 32.44, 32.76, 44.20, 3.72, 7.20 µ moles of phenol liberated/ mg of protein were recorded on tissue of treated fish which represented in Table 1. Moreover, the respective concentration of $Pb(C_2H_3O_2)_2$ exposed against fingerlings which compared with control and experimental group. the percentage of concentration on SDH, LDH, AST, ALT, ACP and ALP of various organs of treated fish were increased as well as decreased in the experimental group then the control group. The mean values of SDH, LDH, AST, ALT, ACP and ALP values of control and Pb(C₂H₃O₂)₂ treated group was compared for their statistical significance at P<0.05. The present study compared with previously published various studies, heavy metal contamination hugely affects the various biological activities. The huge accumulation of Pb were noticed in the various organs of fish: gills 0.151 mg/g and followed by bones 0.108 mg/g as well as minimum level in muscle tissues 0.078 mg/g. Similarly, Cd extremely accrued in the bones 1.750 mg/g, followed gills 0.083 mg/g and muscle tissues 0.004 mg/g (Ahmad et al. 2020). Similar approaches were noticed by the influences of heavy metal PbNO₃ concentration 143.3 mg/l promoted various enzymatic scarcity in O. niloticus fish liver, the various enzymes of AST, ALT, MT, GPx and CAT were unimaginable deficiency occurred by the interaction of PbNO₃ on O. niloticus (Ahmed et al. 2020).

The heavy metal are high toxic agents in all living faunas, the present work evidently supported by earlier published many studied, the heavy metals lead (Pb) and cadmium (Cd) exposed towards Nile tilapia were studied against enzymological and antioxidant activities. The maximum Pb and Cd level in 0.720, 0.114 and 1.137, 0.313 and 1.190, 0.310 ppm in muscles, liver and gills and respectively. Correspondingly, antioxidant activity notably similar observation had occurred in MDA, SOD, GPX, GSH, and GST of Nile tilapia (Alaa Eldin et al. 2021). The Pb toxicity was observed on Nile tilapia, the biological effects of Pb (NO₃)₂ toxicant observed against growth, blood cell and histopathology of various fish organs gills, liver and intestine of Nile tilapia. The all observation were statistically significant in fish behavioral abnormalities. arowth rate.

hepatosomatic index. ervthrocytes counts and mortality rate by the exposure of Pb (NO₃)₂ toxicant in Nile tilapia (Mostafa et al. 2022). The heavy metal toxicant greatly influenced in various levels of biological stress, enzymological deficiency, improper digestive factor (Mahi et al. 2022), lesser blood composition count, histological changes (Shahjahan et al. 2020), delayed nutritional absorption, mortality on aquatic organism (Islam et al. 2022). Similarly, our present study evidently supported by earlier output, the fingerlings Labeo rohita subjected to assess the $Pb(C_2H_3O_2)$ toxicological and histopathological assessment was noticed on liver and kidney, LC₅₀ ranges were 6.86, 3.43 and 1.71 ppm against different 1/5th, 1/10th concentrations 1/20th and respectively (Dawood et al. 2022). The industrial discharges found different heavy metals (Mn, Fe, Pb, Ni, Cr, Hg, As, Zn and Fe) contamination which produced various physiological and biochemical alteration sufficiently increase and decrease the serum biochemical parameters in (AST, ALP, SOD, GPx, CAT and GSH) aquatic fish O. niloticus (Ali et al. 2024). The present study concludes that the sublethal concentration of Pb(C₂H₃O₂)₂ breaks down transaminase activities in the liver, gill, muscles and kidney tissue of Oreochromis mossambicus. The alteration in these enzymes suggests an increased participation of protein in the energy metabolism in response to an increased energy demand to cope with stress situation (Ishaq et al. 2023).

4. CONCLUSION

The $Pb(C_2H_3O_2)_2$ contamination sufficiently influenced in the various enzymological effects on O. mossambicus. It has also been noticed that Pb(C₂H₃O₂)₂ toxicant significantly changing the enzymological deficiency in the different organs (liver, gill, muscles and kidney tissue) of O. mossambicus. Overall, present findings exhibited higher concentrations of $Pb(C_2H_3O_2)_2$ showed a negative impact on different organs of O. mossambicus. The outcome of the study supported the over dosages of $Pb(C_2H_3O_2)_2$ toxicant will exploit the entire fish communities and other faunas also. It is informed that the use and discharge of metals into the environment should be mitigated or should be discharged into the system after proper treatments.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models

(ChatGPT, COPILOT, etc.,) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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