



Lipid Peroxidation and Antioxidant Effects of Lead Acetate: An Experiment 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 industrial wastes are extremely deadliest agents which are heavily settle into the aquatic ecosystems as the results they rising uncountable health defects towards aquatic species. In the present study, the effect of heavy metal Lead acetate ($Pb(C_2H_3O_2)_2$) exposure towards fingerlings of *Oreochromis mossambicus*. The fish were exposed to sub lethal concentration LC_{50} of $Pb(C_2H_3O_2)_2$ for 96 hrs. for 21 and 28 days and the various organs viz. liver, gill, kidney and muscle tissues were studied for lipid peroxidation (LPO), Reduced glutathione (GSH), glutathione peroxidase (GPx), Catalase (CAT), and superoxide dismutase (SOD). These observed mean data were subjected to student 'T' test. The $Pb(C_2H_3O_2)_2$ exposed to *O. mossambicus* fingerlings which tested on various organs of liver, gill, kidney and muscle for assessing the effect of LPO, GSH,

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GPx, CAT and SOD on control and treated fish. The predominant effects were observed at 28 days of exposure period, the liver, gill, kidney and muscle has drastically suffered by exposure of $Pb(C_2H_3O_2)_2$. Liver: 2.89, 2.40, 1.21, 1.33 and 0.42 $\mu\text{mole/mg}$ of protein/hr, Gill: 0.948, 1.18, 0.326, 0.62 and 0.24 $\mu\text{mole/mg}$ of protein/hr, Kidney: 0.968, 1.56, 1.006, 0.46 and 0.26 $\mu\text{mole/mg}$ of protein/hr and Muscle: 0.988, 1.12, 0.226, 0.62 and 0.12 $\mu\text{mole/mg}$ of protein/hr were recorded on tissue of treated fish. 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 LPO, GSH, GPx, CAT and SOD of various organs of treated fish were increased as well as decreased in the experimental group than the control group. The huge exposure of $Pb(C_2H_3O_2)_2$ on *O. mossambicus* fingerlings which showed unpredictable negative health defects on various system. Beyond the threshold level of $Pb(C_2H_3O_2)_2$ it may interfere the various metabolic activity of organism including lipid peroxidation and antioxidant level. Hence, the present experiment which gives the great knowledge about effects of $Pb(C_2H_3O_2)_2$ on aquatic organism as well as health defects on faunal communities by using of higher concentration.

Keywords: $Pb(C_2H_3O_2)_2$; *Oreochromis mossambicus*; various organs; metal toxicant; lipid peroxidation; antioxidant effects.

1. INTRODUCTION

Globally, the various anthropogenic activities: agronomic pesticides and fertilizer wastes, shrimp culture, industrial and household effluents, mining wastes, etc., are having rich amounts of heavy metals as the results the metal contamination may occurred in different states of biosphere including aquatic ecosystem [1], by the extreme accumulation of various heavy metals are significantly destroying the natural system [2]. Heavy metal while entered into the aquatic ecosystem they are eventually dissolved in water and easily settle into the various organs of aquatic faunas especially in fish species [3], they finally entered into predatory/ consumer species including human [4]. The rapid settlement of various pollutants which promoting countless of disorder to both faunal and floral communities [5]. Among the various eco-system, particularly the aquatic ecosystem predominantly receives a wide range of pollutants. The pollutants in aquatic environments which promote unavoidable hazards, disturbs the survival and growth rate, reproduction, biochemical, physiological effects in the various aquatic organisms [6]. Lead acetate ($Pb(C_2H_3O_2)_2$) is a heavy metal which causing many different health defects: skin allergy and irritation, organ and system failure, acute and chronic toxicity as well as countless of reports have investigated towards hazardous capabilities of $Pb(C_2H_3O_2)_2$ in many experimental faunas [7]. $Pb(C_2H_3O_2)_2$ is a prime toxic metal which has been used in different industrial properties and it has hugely abundance in dyes, batteries, insecticides, fuels, and other industrial properties [8]. It extensively exposed by polluted nutrients and aquatic

mediums [9]. They mainly give toxic effect in different internal organs including liver, kidney, nerves system, reproductive and other side effects also [10]. The enzymes are very importance for regular metabolic activities including many organs and it any changes occurred that could be led into many different side effects [11]. The degenerative changes due to the combined metal toxicity exhibited in the liver, gill, kidney and muscle alter level of a number of its enzymes [12]. The enzymes are biomarkers of acute hepatic damages and its bioassay can serve as a diagnostic tool for assessing the functions of the liver, gill, kidney and muscle [13]. Very few works only have reported on aquatic organisms to $Pb(C_2H_3O_2)_2$ exposure. Henceforth, the present study focuses on, heavy metal $Pb(C_2H_3O_2)_2$ on the lipid peroxidation and antioxidant level in the liver, gill, kidney and muscle tissue of *O. mossambicus*.

2. MATERIALS AND METHODS

2.1 Experimental Organism

The selected *O. mossambicus* aquatic fish species were procured carefully (Hale and healthy, even sized, 15day old fingerlings) from East Coastal Zone of Poompuhar Village, Mayiladuthurai District, Tamilnadu, India. The procured fingerlings were carefully transported in air-filled polythene container CI free H_2O . Around 100 nos. fingerlings were kept in each container as well as CI free H_2O well aerated, using pressurized air from a cylinder. This mode of transit proved successful, since there was no mortality in all consignments throughout the course of this study. The procured fingerlings

were maintained separately under laboratory condition. The fingerlings were transferred for acclimatization in large rectangular tanks (100X100X100cm) 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 KMnO_4 solution. The fingerlings were disinfected with 0.1% KMnO_4 solution and were maintained for three weeks in well-aerated Cl free H_2O . to Cl free H_2O 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 23°C for 15 days as well as maintained all physiochemical parameters.

2.2 Experimental Design

Fish were selected for the experiment from the stock irrespective of the sex. The size selected for the experiments were 80-100mm length and 5-10g of weight fish were divided into two equal groups each comprising of 20 fishes. Each group was kept in separate plastic trough. The first groups were kept as control and were maintained in normal water without any treatment. The second group was exposed to a sub-lethal concentration of 96 hrs. LC_{50} of $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2$ for 21 and 28 days. Solution was renewed once in 24hrs. exposure period. The fish from the respective experimental as well as control groups were sacrificed and liver, gill, muscle and kidney tissue were isolated from the fish and used for the estimation lipid peroxidation and antioxidant parameters.

2.3 Estimation of Lipid Peroxidation and Antioxidants

Lipid peroxidation (LPO): The selected organs were isolated and removed carefully from treated and control fish, organs were homogenate neatly and prepared in Tris-HCl buffer (pH 7.5). 1.0 ml of homogenate was kept in cleaned test tube add 2.0 ml of TBA-TCA-HCl reagent was mixed thoroughly. The composite was kept in a boiling water bath (60°C) for 15 minutes. After cooling the composite, the pink-coloured chromophore was read at 535 nm against the reagent blank in UV spectrophotometer. 1, 1, 3, 3- tetramethoxy propane was used to construct the standard graph. The values were expressed as n moles/mg wet wt. of tissue followed by the standard method [14].

Reduced glutathione (GSH): The selected organs were isolated and removed carefully which homogenized in PO_4^{3-}

buffer and centrifuged at 2500 rpm for 5 minutes. Supernatant 0.2 ml kept in test tube with 1.8 ml of EDTA along with 3.0 ml of precipitating reagent. The composites mixed thoroughly, kept for 5 minutes and centrifugation at 3000 rpm for 10 minutes. Centrifugated aliquot was filtered, 2.0 ml taken and added 4.0 ml of 0.3M Na_2HPO_4 solutions along with 1.0 ml of DTNB reagent were added. The appearance of yellow colour was read at 412 nm in UV spectrophotometer which construct the standard graph followed by followed by the standard method [15].

Glutathione peroxide (GPx): The organs were homogenized in PO_4^{3-} buffer, centrifuged at 2,500 rpm for 5 minutes, 0.2 ml of clear supernatant taken added with enzyme mixture (0.2 ml of PO_4^{3-} buffer, 0.2 ml 0.4 nM of EDTA, and 0.1 ml of NaN_3) in a test tube. This composite mixed well, kept for 2 minutes for 37°C in an incubator. After the incubation period, 0.2 ml of GPx and 0.1 ml of H_2O_2 were again added to the above mixture and incubated at 37°C exactly for 10 minutes. The reaction was arrested by the addition of 0.5 ml of 10% TCA. The colour was developed and then read at 412 nm. They construct the standard graph followed by the standard method [16].

Catalase (CAT): The organs were homogenized by PO_4^{3-} buffer which centrifuged for 10 minutes at 2000 rpm. In a clean test tube, 0.9 ml of PO_4^{3-} buffer, 0.1 ml homogenate and 0.4 ml of H_2O_2 were added with 2 ml of $\text{C}_2\text{H}_4\text{Cr}_2\text{O}_9^{2-}$. The composites tubes were kept in water bath at 37°C for 10 minutes and then allowed to cool. The colour developed was read at 620 nm in UV-spectrophotometer. H_2O_2 was used to construct the standard graph followed by the standard procedure [17].

Superoxide dismutase (SOD): The organs homogenized in 2 ml of 0.25 M $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ solution and centrifuged at 10,000 rpm in cold condition for 30 minutes. The supernatant was dialyzed against Tris-HCl buffer (0.0025 M, pH 7.4). The supernatant obtained was used as enzyme source. One unit of enzyme activity is defined as the enzyme reaction which generates 50% inhibition of NBT reduction in one minute under the assay condition and these values are expressed as unit/min/mg protein. Each unit is the amount of tissue that inhibits 50 % reduction of NBT and the test was assessed by the following method [18].

3. RESULTS AND DISCUSSION

The selected concentration of heavy metal $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2$ exposed to fingerlings of

Table 1. The observation of lipid peroxidation and antioxidants level in various organs of *O. mossambicus* by the exposure of $Pb(C_2H_3O_2)_2$ heavy metal

Various organs	Parameters	Control	Exposure 21 days	% Change	Exposure 28 days	% Change
Liver	LPO	1.23±0.24	2.74±0.39	43.61	2.89±0.76	82.29
	GSH	6.78±0.22	3.29±0.27	-33.60	2.40±0.39	-30.22
	GPx	1.98±0.64	1.35±0.31	-16.30	1.21±0.62	-22.38
	CAT	3.58±0.24	1.64±0.28	-30.24	1.33±0.21	-41.80
	SOD	1.56±0.28	0.65±0.26	-33.29	0.42±0.44	-45.28
Gill	LPO	0.234±0.22	0.856±0.34	38.49	0.948±0.89	53.64
	GSH	1.98±0.46	1.42±0.86	-12.39	1.18±0.46	-21.78
	GPx	0.988±0.26	0.422±0.36	-18.50	0.326±0.22	-28.63
	CAT	1.82±0.44	0.82±0.25	-38.22	0.62±0.42	-42.28
	SOD	0.95±0.02	0.42±0.48	-21.08	0.24±0.20	-36.90
Kidney	LPO	0.289±0.25	0.856±0.28	24.62	0.968±0.22	53.28
	GSH	2.86±0.24	2.08±0.42	-22.39	1.56±0.63	-38.74
	GPx	1.856±0.21	1.084±0.12	-16.81	1.006±0.22	-24.40
	CAT	1.92±0.42	1.32±0.20	-42.60	0.46±0.28	-48.32
	SOD	0.88±0.44	0.51±0.28	-31.06	0.26±0.77	-42.06
Muscle	LPO	0.244±0.32	0.824±0.37	38.44	0.988±0.32	45.28
	GSH	1.68±0.04	1.21±0.24	-18.60	1.12±0.28	-22.46
	GPx	0.86±0.021	0.322±0.72	-12.48	0.226±0.08	-17.12
	CAT	1.46±0.22	0.72±0.02	-25.44	0.62±0.76	-37.66
	SOD	0.64±0.20	0.20±0.40	-32.28	0.12±0.44	-39.62

The data were statistically evaluated into Mean ± Standard Error

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

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

LPO: Lipid peroxidation ($\mu\text{mole/mg}$ of protein)

GSH: Glutathione ($\mu\text{mole/mg}$ of protein)

GPx: Glutathione peroxidase ($\mu\text{moles/mg}$ protein)

CAT: Catalase (Unit/mg of protein)

SOD: Superoxide dismutase (Unit/mg of protein)

O. mossambicus which tested on various organs such as liver, gill, kidney and muscle in various interval periods for assessing treatment and control against lipid peroxidation and antioxidants level. The major effects were noticed at 28 days of exposer tenure, the various organs (liver, gill, kidney and muscle) of *O. mossambicus* had severely affected by exposer of $Pb(C_2H_3O_2)_2$. Liver: 2.89, 2.40, 1.21, 1.33 and $0.42\mu\text{mole/mg}$ of protein/hr, Gill: 0.948, 1.18, 0.326, 0.62 and $0.24\mu\text{mole/mg}$ of protein/hr, Kidney: 0.968, 1.56, 1.006, 0.46 and $0.26\mu\text{mole/mg}$ of protein/hr and Muscle: 0.988, 1.12, 0.226, 0.62 and $0.12\mu\text{mole/mg}$ of protein/hr were observed on tissue of $Pb(C_2H_3O_2)_2$ treated fish. Moreover, the respective concentration of $Pb(C_2H_3O_2)_2$ exposed against fingerlings which compared with control (without treated $Pb(C_2H_3O_2)_2$) and experimental group, the percentage of LPO, GSH, GPx, CAT and SOD level in various organs of treated fish were increased as well as decreased in the

experimental group then the control group and it is represented in Table 1 as well as similar observation also noticed in 21 days of exposure period. The mean values of LPO, GSH, GPx, CAT and SOD values of control and $Pb(C_2H_3O_2)_2$ treated group was compared for their statistical significance at $P < 0.05$. Previously, the similar kind of study reported, the SiO_2 nanoparticles at 5 mg/L for 96 hrs. showed the various biological changes in the liver tissues, by the exposure they were changed the disorganized hepatic parenchyma, vacuolization and disintegrated nucleus, cytoplasmic vacuolization and leukocyte infiltration [19]. These lipid peroxides and hydroxyl radicals may cause outer skin damages and which led into destroy the entire cell membrane and its contents [20]. In the present work, the $Pb(C_2H_3O_2)_2$ exposed to several hours of aquatic organism *O. mossambicus* fish which drastically reduced the free radical scavenger enzymes GPx, CAT, and SOD. The drastic

destruction of GPx, CAT and SOD (free radical scavenger enzymes) by the direct influences of $Pb(C_2H_3O_2)_2$ sublethal concentration on concern fish species *O. mossambicus*. The higher concentration of heavy metal directly interrelates with internal organ tissues which considerably reduced the enzyme secretion and its activities [21]. The $PbNO_3$ exposed to *O. niloticus* fingerlings with their LC_{50} values were 143.3 mg/l for *O. niloticus* as well as which drastically suppressed counts of RBCs, Hb, PCV, MCV, MCH, MCHC, AST and ALT deterioration of hepatic tissue. The $PbNO_3$ toxicity on *O. niloticus* were observed the changing of antioxidant enzymes level GPx, and CAT in hepatic tissue [22]. The industrial wastes highly suffering the aquatic organisms which constrict of various heavy metals Mn, Fe, Pb, Ni, Cr, Hg, As, Zn and Fe. They were seriously affected the bio-system of *O. niloticus* fish population, the Mn and Fe LC_{50} values were 147.36mg/L and 90.52mg/L, respectively [23]. Correspondingly, similar kind of reports were observed from previous studies [24], the heavy metal and various causative agents considerably various biological and enzymological (AST and ALT level) changes occurred in the freshwater fish *O. niloticus* [25], *C. cirrhosis* [26] *D. rerio* [27]. It can be stated that the different interval exposure of $Pb(C_2H_3O_2)_2$ affects the various organs of *O. mossambicus* for observing lipid peroxidation and antioxidant responses under laboratory conditions.

4. CONCLUSION

$Pb(C_2H_3O_2)_2$ is a high toxic metal in many of the faunal species which are considerably making many hazards to environment as well as living species. The huge exposure of $Pb(C_2H_3O_2)_2$ may cause the various health issues as well as metabolic activity also. This study may give the awareness about exposure of heavy metal in various eco-system and it could be given the great knowledge about metal contamination in aquatic fauna and various health defects on faunal communities by using of higher concentration.

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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