ejpmr, 2020,7(2), 354-361

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

LEAD ACETATE INDUCED TOXICITY ON LIPID METABOLITES IN DIFFERENT PARTS OF *DANIO RERIO* (ZEBRA FISH)

Syeda Viquar Unnisa[1], Dr. Ghousia Begum[2]*, Khwaja Amtul Raouf Qazi[1]

[1]Department of Pharmacology, Sultan Ul Uloom College of Pharmacy, Hyderabad, Telangana, India 500034.
[2]Department of Toxicology, CSIR-IICT, Hyderabad, Telangana, India 500007.

***Corresponding Author: Dr. Ghousia Begum**

Department of Toxicology, CSIR-IICT, Hyderabad, Telangana, India 500007.

Article Received on 12/12/2019 **Article Revised on 02/01/2020** **Article Accepted on 23/01/2020**

ABSTRACT

Aquatic toxicology refers to impact of natural materials and manufactured chemicals on marine sources. Metal contamination is widely spread particularly in regions influenced by anthropogenic and industrial activities. The acute toxicity study (LC_{50}) of lead acetate to *Danio rerio* (zebrafish) at 96 hrs is determined as 354.8 ppm. The sub – lethal concentration 118.26 ppm of lead acetate is selected to carry out the biochemical estimations in tissues of zebrafish for 4 days by using standard methods. The results of the study indicated decrease in total lipids and increase in free fatty acid and cholesterol in gills, viscera and body of fish. The behavioral patterns such as irregular, erratic jerky swimming movement around the experimental aquaria, followed by drowning down has been observed during the exposure period. Therefore, measurement of biochemical parameter is considered to be significant tool in toxicology to diagnose the health status of fishes in the aquatic ecosystem.

KEYWORDS: Aquatic toxicology, lead acetate, *Danio rerio*, LC_{50}, biochemical parameters.

INTRODUCTION

Aquatic toxicology is study of impacts of natural materials, manufactured chemicals and anthropogenic activities on marine organisms at different organization levels from sub - cellular to various communities and ecosystems.[1] The universal issue today is environmental pollution and the most significant pollutants in aquatic system are heavy metals because of their accumulation and bio-magnification by marine animals producing toxicity. Heavy metals are taken up by fish into their bodies either through ingestion via alimentary tract or gills and skin.[2] Effectively after the absorption, metals within the fish are circulated through the blood to tissues and organs where they get accumulated.[3] Fish accumulate toxicants/pollutants favorably in liver and the biological effects are observed when metal concentrations in tissues reach a threshold level.[4] However, this accumulation relies upon ingestion, storage and excretion of metal from their body.[5] This concludes that fish with more intakes and less rate of metal excretion are said to be have higher levels of accumulation.[6] Lead is one of the most toxic metals for aquatic organisms. The metal content exceeding threshold concentration can be toxic to the cell as metal ions act as oxidants that bind to biomolecules. These metal ions react with sulfhydryl groups of protein by displacement of metals from their original binding sites producing antagonistic action against cells that ultimately leads to toxicity. As reports on developmental toxicity of zebrafish are plentiful,[7,8] the present study is taken up to investigate the sub - lethal toxicity of Pb on certain lipid metabolites, total on adult zebrafish (*Danio rerio*). As gills play characteristic role in osmotic regulation and oxygenation of blood [9] hence, gill tissue along with viscera and body of fish was selected for sub-acute studies.

MATERIALS AND METHODS

All the reagents used in the current research study are of analytical grade. The test compound 'Lead Acetate Trihydrate' was purchased from Merck Life Sciences Pvt. Ltd., India and was 99.5% of purity. The fish, *Danio rerio* were collected from Chennai fish farm, which is relatively free from pollutants and were brought to the laboratory in large aerated bags. Later, they were acclimatized in laboratory for 15 days in glass aquaria (60 x 30 x 30 cm) and fed daily with (TAIYO). The average values for water quality data in exposure tanks is as follows pH 7.10±0.05, dissolved oxygen 8.24 ±0.22 mgL^{-1}, temperature 24±2° C, chlorides 245.57±1.44 mgL^{-1}, total hardness 415±1.2 mgL^{-1}, Alkalinity 348±1.6 mgL^{-1} (as CaCO3).

Acute Toxicity

Determination of LC_{50}

The LC_{50} value of lead acetate was determined in the laboratory using semi-static method (UNEP).[10] The required concentrations 300, 325, 350, 375 and 400 ppm were maintained in 5 litres of water by mixing the toxicant into the water and renewed daily. A parallel

control experiment without toxicant was also performed. Healthy and similar weight fish with one day starved (10 per aquarium) were exposed to the above mentioned concentrations with a minimum of two replicates. The fish starved during 96 hrs of exposure period. The mortality was recorded in each test concentration was recorded after 24 hrs for a period 96 hrs. The test concentration, percent mortality and the number of fish per concentration determined the median lethal concentration (LC_{50}) by the method of Finney.[11]

Sub Acute Toxicity

Sub-acute study is the measure of the biological activity of the compound and provides a relative estimate of the lowest dosage required to produce the effect following repeated exposure. In the present study the sub - lethal concentration of lead acetate i.e., 118.26 ppm is selected to carry out biochemical parameter in tissues of zebra fish. Thirty healthy and one day starved fish were categorized into two groups one is control and other is experimental. The experimental group is exposed to 118.26 ppm of toxicant for 4 days. The water renewal was done for every 24 hrs and fresh toxicant was added in order to maintain the concentration of toxicant constant. At the end of 1, 2, and 4 days five fish each from controls and exposed groups were taken and sacrificed to isolate the gills, viscera and body. The biochemical parameters, total lipids, cholesterol and free fatty acids were estimated in these tissues.

Determination of Total Lipids

Lipids were extracted as described by Folch et al [12] and estimated by the method of Barnes and Black stock [13]. Tissues (gills, viscera and body) were homogenized (5% w/v) in a waring blender in chloroform-methanol mixture (2:1). The homogenates were filtered through Whatman No. 1 filter paper which is rinsed with solvent and the residue was rehomogenized as before and then filtered. The non-lipid matter from pooled filtrate was removed by shaking vigorously with 0.88% KCI (added as one fourth of the volume). 1 ml of filtrate was taken in a test tube and evaporated under nitrogen and 1 ml of concentrated H_2SO_4 was added and boiled for 10 min. For estimation of total lipid 0.2 ml of solution was taken and 5 ml of vanillin reagent was added. The developed color was read in spectrophotometer at 520 nm against reagent blank.

Determination of Cholesterol

Cholesterol was estimated by the Zarrow et al,[14] method. Homogenize the tissues (gills, viscera and body) in the 5%w/v of ethanol: ether (1: 1) solvent mixture and centrifuged for 10 minutes at 3000 rpm. 1 ml of supernatant is taken and 2 ml of glacial acetic acid and coloring reagent is added from sides of test tubes. The two layers are formed, a light brown color appear which changes to purple. Cool tubes to room temperature. Measure the OD at 560 nm along with reagent blank.

Determination of Free Fatty Acids

Free fatty acid was estimated by the method of Ragauw et al.[15] In chloroform: heptane: methanol mixture (220:225:105) 5% (w/v) homogenate of tissues (gills, viscera and body) was prepared and centrifuged for 5 min at 4000 rpm. 5 ml of supernatant was taken in a centrifuge tube and 0.5 ml of copper triethanolamine reagent was added and shaken vigorously and horizontally. The mixture was centrifuged for 5 min at 500 rpm. To 1.5 ml of supernatant, 3 ml of chloroform: heptane: methanol mixture and 0.75 ml of diethyl dithiocarbonate reagent was added and absorbency was read at 435 nm spectrophotometer using chloroform as blank.

Table 1: Lethal concentration of lead acetate to fish (*Danio rerio*).

Concentration	% mortality	log C	Probits(mortality)
300	10	1.322	3.72
325	25	1.3424	4.33
350	45	1.352	4.87
375	60	1.361	5.25
400	75	1.371	5.52

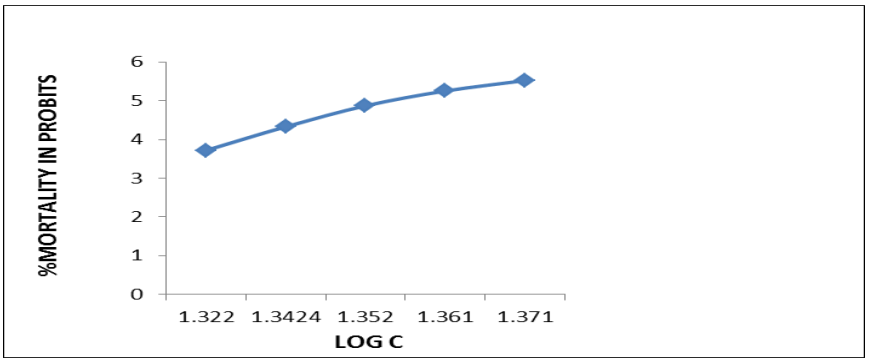

Figure 1: Graph showing 96hrs LC50 linear relationship between probit mortality (%) and log concentration (mgL^{-1}) of *Danio rerio* to various concentrations of lead acetate.

Regression equation (log): Y=ax +c =5=15.48 x -34.58 = 354.8 ppm

Table 2: Tabular and graphical representation of estimation of total lipids in zebrafish tissues exposed to sub-lethal concentration for 4 days.

Tissues	Exposure period (days)			
		1	2	4
Gills	Control	70.30	70.32	70.32
	Exposed	56.90	53.16	45.57
	%Variation	-19.08	-24.5	-35.1
Viscera	Control	316.18	316.15	316.18
	Exposed	279.86	229.0	197.25
	%Variation	-11.48	-27.5	-37.61
Body	Control	201.75	201.70	201.75
	Exposed	188.7	135.25	101.54
	%Variation	-6.4	-32.9	-49.7

Each value is the mean of five individual fish (mg/g wet weight of tissue).

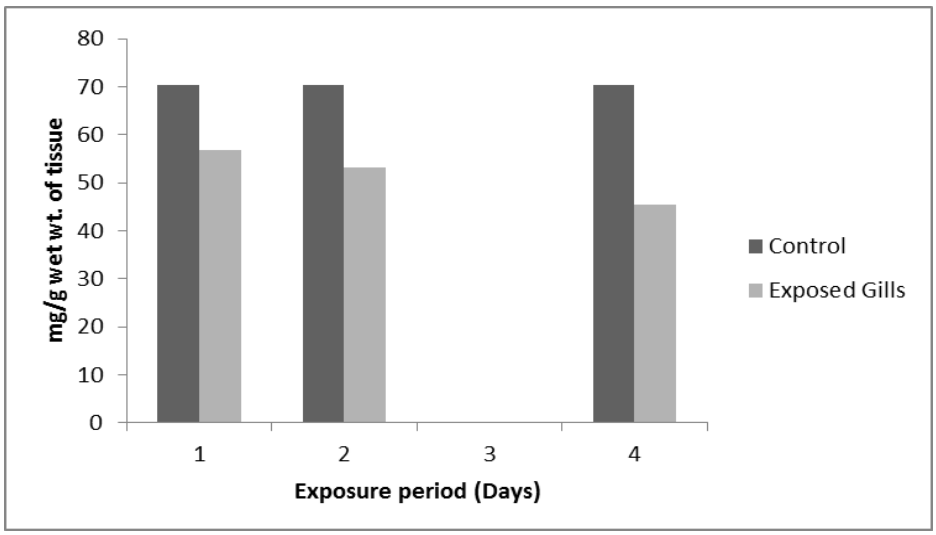

Figure 2: Graph showing % decrease in total lipids in gills of zebrafish.

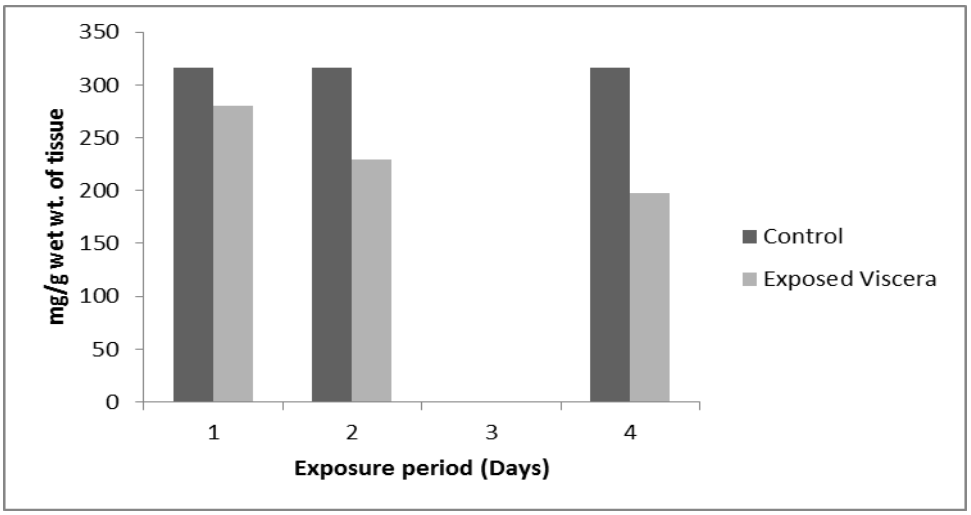

Figure 3: Graph showing % decrease of total lipids in viscera of zebrafish.

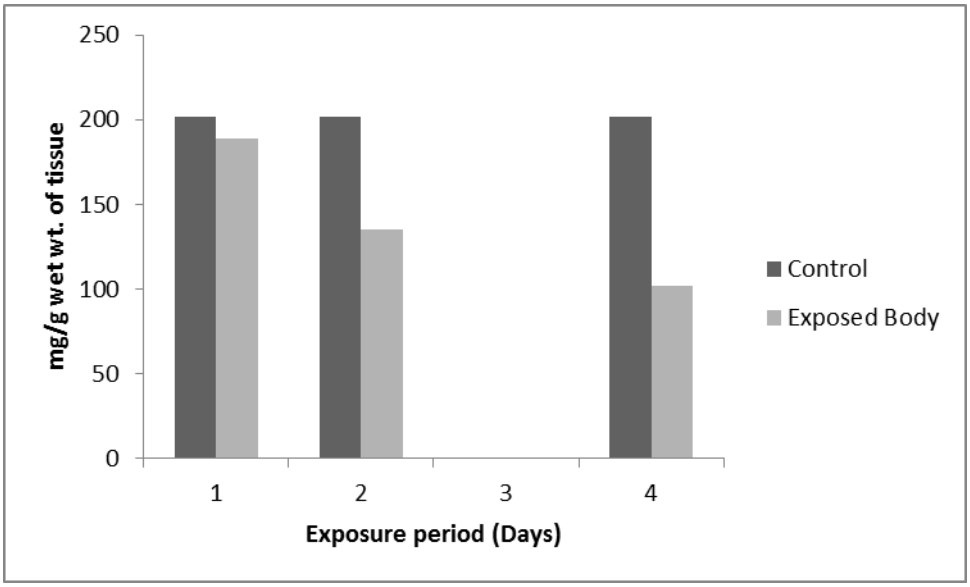

Figure 4 Graph showing % decrease of total lipids in body of zebrafish.

Table 3: Tabular and graphical representation of estimation of Cholesterol in tissues of zebrafish exposed to sub-lethal concentration for 4 days.

Tissues	Exposure period (days)			
		1	2	4
Gills	Control	3.09	3.11	3.11
	Exposed	4.09	4.94	6.01
	%Variation	+32.3	+58.8	+93.2
Viscera	Control	4.50	4.50	4.48
	Exposed	5.60	6.22	6.91
	%Variation	+24.4	+38.2	+53.5
Body	Control	3.31	3.30	3.31
	Exposed	4.2	4.85	5.45
	%Variation	+26.8	+46.5	+64.6

Each value is the mean of five individual fish (mg/g wet weight of tissue).

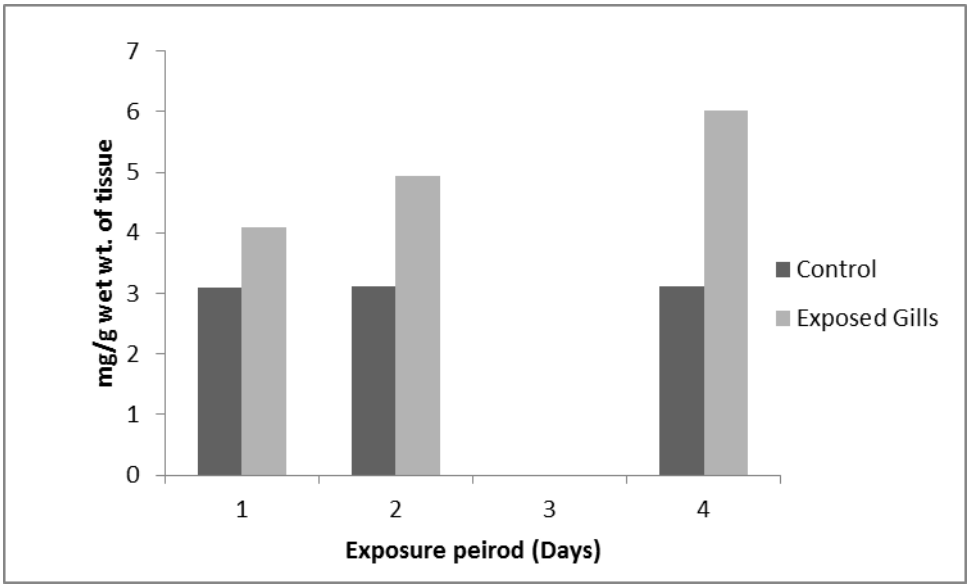

Figure 5: Graph showing % increase of cholesterol in gills of zebrafish.

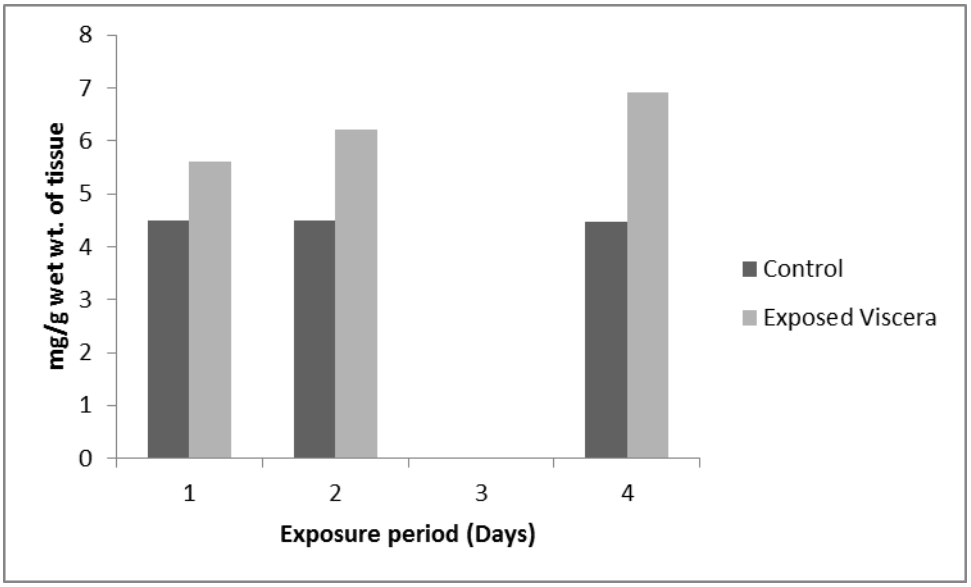

Figure 6: Graph showing % increase of cholesterol in viscera of zebrafish.

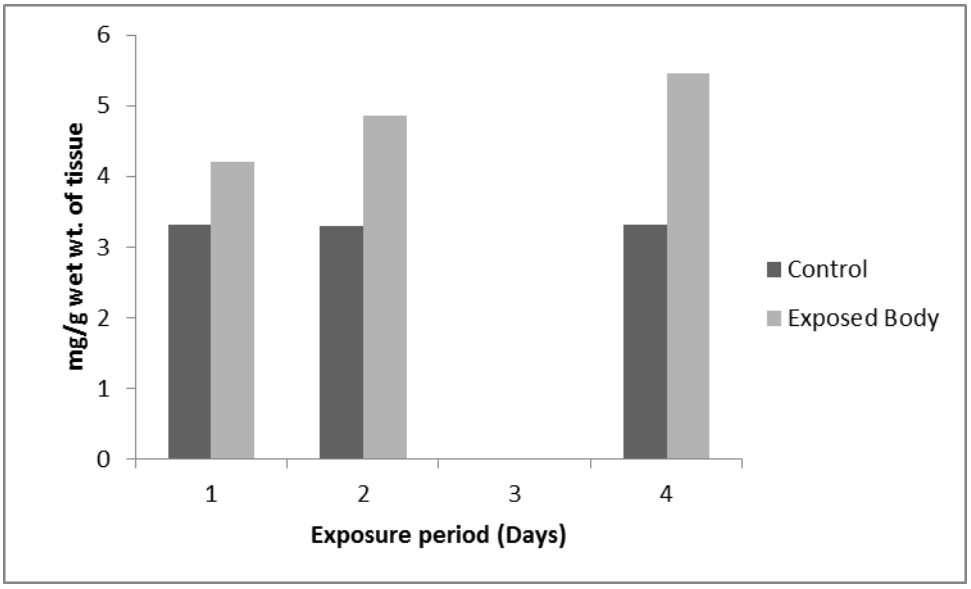

Figure 7: Graph showing % increase of cholesterol in body of zebrafish.

Table 4: Tabular and graphical representation of estimation of Free Fatty Acid in tissues of zebrafish exposed at sub-lethal concentration for 4 days.

Tissues	Exposure Period (Days)			
		1	2	4
Gills	Control	1.20	1.22	1.22
	Exposed	2.25	2.06	1.58
	%Variation	+29.5	+68.8	+84.4
Viscera	Control	1.32	1.34	1.34
	Exposed	1.47	1.83	2.13
	%Variation	+9.84	+36.5	+58.9
Body	Control	2.19	2.18	2.19
	Exposed	2.54	2.78	3.29
	%Variation	+15.9	+26.9	+50.2

Each value is the mean of five individual fish (mg/g wet weight of tissue).

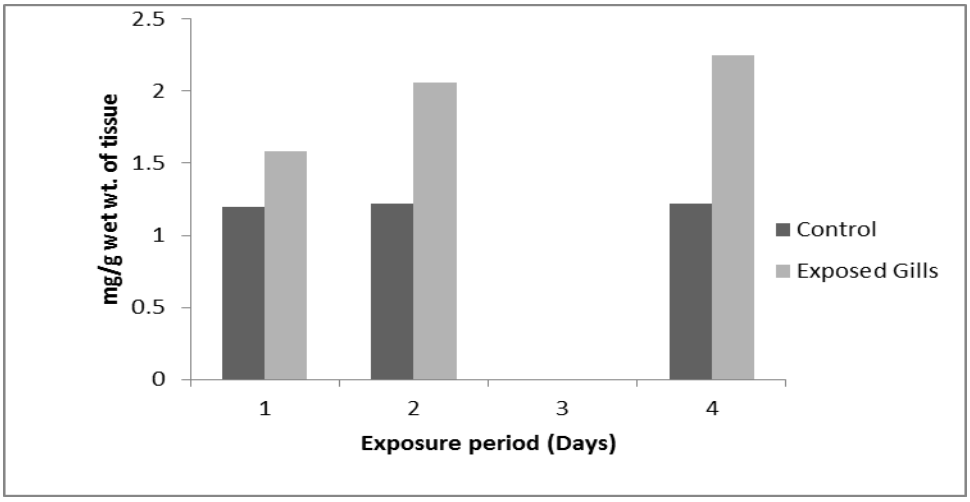

Figure 8: Graph showing % increase of free fatty acid in gills of zebrafish.

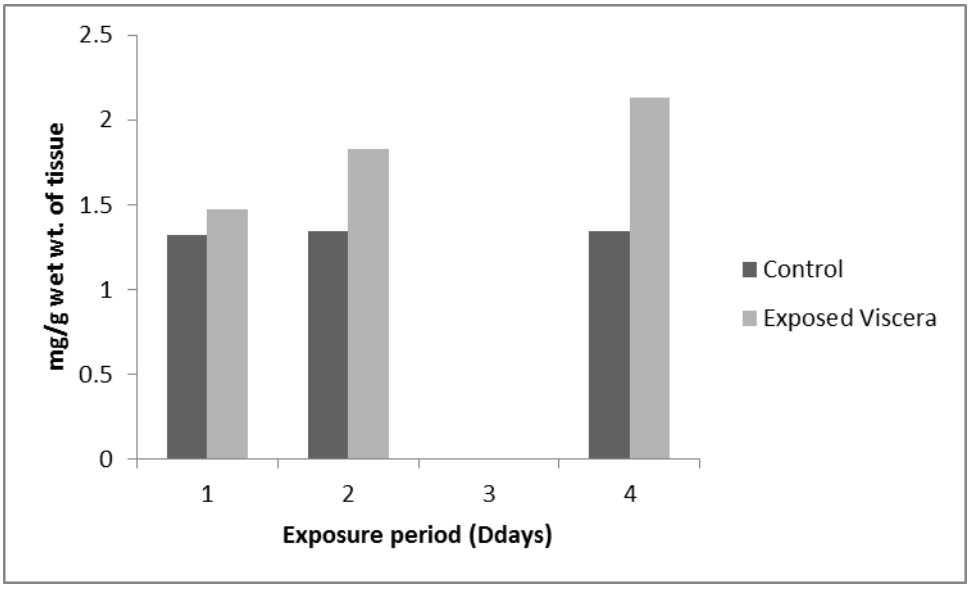

Figure 9: Graph showing % increase of free fatty acid in viscera of zebrafish.

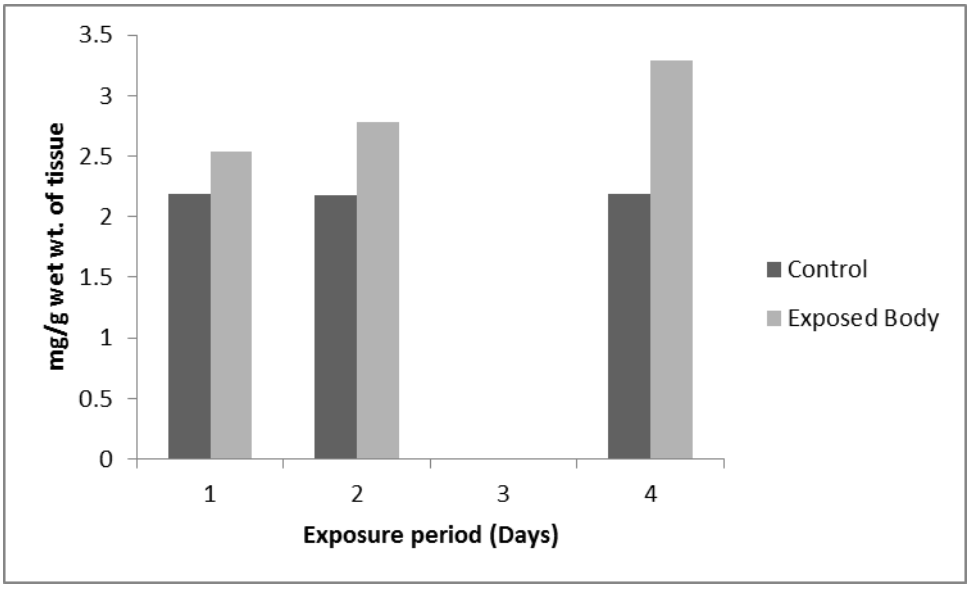

Figure 10: Graph showing % increase of free fatty acid in body of zebrafish.

RESULTS AND DISCUSSION

The LC_{50} value of lead acetate to zebrafish for 96 hrs is determined as 354.8 ppm (figure 1). The fish were exposed to 1/3rd of LC_{50} i.e., 118.26 ppm for 4 days. The total lipids in gill on day one decreased as compared with controls. Decrease in percent (%) continued till the end of exposure period, 4th day. Similar pattern of decrement was observed in viscera and body (Table 2). Cholesterol increase is exposure dependent i.e. with time of exposure increase was also noticed in gills, viscera and body (Table 3). Free Fatty Acid concentration increased in all tissues with percent variation. The % increase is 29.5 in gills; 9.84 viscera and 15.9 in body (Table 4).

Heavy metals are one of the environmental stressors, may alter the biochemical parameters of aquatic organisms, including fish [16]. In the present study the sub lethal concentration of toxicant, lead acetate was selected to expose the fish for 4 days and determination of total lipids, cholesterol and FFA were performed in tissue such as Gills, Viscera and Body. The decrease in levels of lipids and increased in free fatty acids which are formed by breakdown of lipids showed utilization of energy to counteract the toxic effects provoked by the metal. In every organism, glucose, proteins, cholesterol and lipids act as primary energy sources to perform various body functions. Due to heavy metal toxicity, the depot energy source is affected in exposed organisms as per control. The report of many investigators [16, 17] shows hypercholesterolemia in the metal exposed fishes. Similar results have been reported in the present study. The concentrations of cholesterol and its precursors may increase due to the liver failure causing more production of cholesterol in the fish body parts. Kori-Siakpere et al [18] showed reduced lipoprotein lipase activity to be the cause for elevated levels of cholesterol in *Clarius garipinus*. Katti and Sathyanesan [19] also reported decrease in the lipid levels of *Clarias batrachus* when exposed to cadmium. Tulasi et al [20] observed that exposure of fish *Anabas testudineus* to a sub - lethal concentration of lead nitrate for a period of 30 days during its annual reproductive cycle in preparatory phase reduced the total lipids. Although glucose-6-phosphate dehydrogenase and NADPH is not estimated in the present study the decrement of total lipid might also be due to decreased activity of glucose-6-phosphate as suggested by Singh [21]. Lead metal might have caused oxidative stress resulted in high concentration of ROS which would have cause morphological damage at cellular level to cells, lipids, proteins and nucleic acids resulting in a stressed condition [22]. Behavioral changes are the physiological responses shown by the animal which are sensitive indicator of chemically induced stress in aquatic organisms. Since fishes breathe in water they live, changes in the chemical properties of water are reflected in the behavioral activity of animals. Behavioral change is considered as a promising tool in ecotoxicology. The behavioral patterns of zebrafish were regularly monitored during determination of lethal concentration. The fish showed the symptoms of restlessness, swimming impairments like irregular, erratic jerky movements followed by drowning down around the experimental aquaria and fast opercula movements. The result showed decrease in total lipids and increase in free fatty acids and cholesterol concentration in gills, viscera and body of fish.

CONCLUSION

The results of present study on lead acetate toxicity indicate that contamination of aquatic sources by heavy metals can interrupt with the health of fish. Hence, toxicological evaluations are highly required to assess the toxic impact of heavy metals on non target living organisms. Therefore, zebrafish became an ideal experimental model for eco-toxicological studies. The given data can be useful for further heavy metal particularly lead toxicity studies on fish. This implies that necessary precautions must be regulated to protect the aquatic ecosystem. In addition to this, water quality should also be maintained by regulating water quality guidelines and avoiding the contamination of water sources with lead is utmost important for the protection of aquatic biota. Therefore, biochemical parameters are diagnostic tool in toxicology to examine the health status of fishes.

ACKNOWLEDGEMENT

I take this opportunity to express my profound gratitude and deep regard to my guide Dr. Ghousia Begum, Principal Scientist and Associate Professor, AcSIR, Applied Biology Division for her exemplary guidance, monitoring and encouragement throughout the course of this work.

REFERENCES

1. Rand, Gary M, Petrocelli and Sam R. Fundamentals of aquatic toxicology: Methods and applications.1985; Washington: Hemisphere Publishing. ISBN0-891163824.
2. Sfakianakis D.G, Renieri E, Kentouri M and Tsatsakis A.M. Effect of heavy metals on fish larvae deformities: A Review. Environ. Res., 2015; 137: 246–55.
3. Fazio F, Piccione G, Tribulato K, Ferrantelli V, Giangrosso G, Arfuso Fand Faggio C. Bioaccumulation of heavy metals in blood and tissue of striped mullet in two Italian Lakes. J. Aquat. Anim. Health, 2014; 26(4): 278-84.
4. Omar W.A, Saleh Y.S and Marie M.A.S. Integrating multiple fish biomarkers and risk assessment as indicators of metal pollution along the Red Sea coast of Hodeida, Yemen Republic. Eco. Toxicol. Environ. Saf., 2014; 110: 221-31.
5. Abdallah M.A.M and Morsy F.A.E. Persistent organo chlorine pollutants and metals residues in sediment and freshwater fish species cultured in a shallow lagoon. Egypt. Environ. Technol, 2013; 34(13-16): 2389-99.

6. Idriss AA and Ahmad AK. Heavy metal concentrations in fishes from Juru River, estimation of the health risk. Bull. Environ. Contam. Toxicol, 2015; 94: 204-08.
7. Nicole M. Roy, Sarah DeWolf and Bruno Carneiro. Evaluation of the Developmental Toxicity of Lead in the *Danio rerio* Body. Aquat. Toxicol, 2015; 158:138-48.
8. Miao W1, Zhu B2, Xiao X3, Li Y1, Dirbaba NB1, Zhou B4 and Wu H5. Effects of titanium dioxide nanoparticles on lead bioconcentration and toxicity on thyroid endocrine system and neuronal development in zebrafish larvae. Aquat. Toxicol, 2015; 161: 117-26.
9. Roberts R. J and Ellis A. E. The anatomy and physiology of teleosts. In Fish Pathology (R. J. Roberts, ed.) 3rd ed. 2001; 12–54. Philadelphia, USA: W. B. Saunders.
10. UNEP 1989 Test of the acute lethal toxicity of pollutants to marine fish and invertebrates: Reference Methods for Marine Pollution Studies No.43, UNEP.
11. Finney D. J. Statistical Treatment of the Sigmoid Response Curve. Cambridge University Press, London, 1964; 20.
12. Folch J, Iers M. and Stanley G. H. S. J. Biol. Chem., 1957; 226: 497.
13. Barnes H and Black Stock J. J. Expl. Mar. Biol. Ecol., 1973; 12: 103.
14. Ragauw B. J. M, Cornelissen P. J. H. C, Helder R, Spikas A. P, Yuonne J. B. F. and Weeber M. M. Clin. Chem. Acta, 1971; 31: 187.
15. Zarrow M.X, Yochim J.M. and Mc Carthy J.L. Experimental endocrinology: A source book of basic techniques. 1964. Academic Press, New York.
16. Yang J.L and Chen H.C. Effects of Gallium on common carp *Cyprinus carpio*: acute test, serum biochemistry and erythrocytes morphology. Chemosphere, 2003; 53(8): 877-82.
17. Singh S.K and Reddy D.V. Subcliniccal hypothyroidism and risk of hypercholesterolemia. Ann. Clin. Biochem, 1990; 27(2): 110-13.
18. Kori-Siahpere O, Ikomi R.B. and Ogbe M.G. Biochemical Response of the African Catfish *Clarias gariepinus* to Sub-Lethal Concentrations of Potassium Permanganate. Ann. Of. Bio. Res., 2011; 2: 1- 10.
19. Katti S.R and Sathyanesan A.G. Changes in tissue lipid and cholesterol content in the fresh water cat fish *Clarius batrachus* exposed to cadmium chloride. Bull. Environ. Contam. Toxicol, 1984; 32: 486-90.
20. Tulasi S.J, Reddy P.V and Rao J.V. Accumulation of lead and effects on Total Lipids derivative in fresh water fish *Anabas testudineus*. Ecotoxicol. Environ. Saf., 1992; 23(1): 33-38.
21. Singh V.P. Effect of some insecticides on liver, blood serum and gonads on fresh water teleost *Channa punctaus*. A biochemical study PhD thesis. Punjab Agriculture University, Ludhiana., 1985; 64-135.
22. Mathew B. B, Tiwari A and Jatawa S. K. Free radicals and antioxidants: A review. J. Pharm. Res., 2011; 4(12): 4340–43.