Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.4055

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **5 (5):** 828-832 (2017)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Toxic Effect of Lead Nitrate on Lipid Peroxidation and Protein Carbonyl Content in the Muscle of Freshwater Fish *Channa striatus* (Bloch, 1793)

Shivani Sharma[1*], Vipin Vyas[2] and Sadhna Tamot[3]

[1]Department of Zoology and Applied Aquaculture, Barkatullah University, Bhopal (M.P)

[2]Department of Biosciences, Barkatullah University, Bhopal (M.P)

[3]Department of Zoology, Sadhu Vaswani College, Bairagarh (M.P)

*Corresponding Author E-mail: ss.shivani001@gmail.com

Received: 15.06.2017 | Revised: 23.07.2017 | Accepted: 6.08.2017

ABSTRACT

Lead nitrate is one of the most common heavy metal that effect adversely on fresh water fishes. In the present study an attempt has been made to find out the mechanism of adverse effects of lead nitrate in fresh water fish, Channa striatus after exposure to various concentrations of lead nitrate. We measured changes in lipid peroxidation and protein carbonyl content in the muscle of C. striatus as a marker of oxidative stress after exposure to different concentrations (8, 18 and 28 mg/l) of lead nitrate for a period of 30, 60 and 90 days. Our observations showed a significant increase (P<0.05) in lipid peroxidation and protein carbonyl content with increase in concentration of lead nitrate at various time intervals in the muscle of fish. Results of the present study revealed an increase in oxidative stress in the muscle of C. striatus with increased concentration of lead nitrate and duration of exposure.

Key words: *Lead nitrate, Muscle, C. striatus, Lipid peroxidation, Protein carbonyl content.*

INTRODUCTION

Contaminants emerge as a major researchable issue that can cause severe effects in the environment[1]. Heavy metal contaminants pose a serious environmental threat because of their persistence and toxicity in aquatic ecosystems[2]. Heavy metals like copper, lead, cadmium, mercury etc. are reported to be present in aquatic ecosystem and create a number of health hazards in aquatic organisms resulting in great loss to fish production[3]. In aquatic environment, many xenobiotics cause oxidative stress in organisms which leads to the production of reactive oxygen species and change in the antioxidant defense system[4] thereby causing damage in the membrane of lipids and proteins. Several studies showed higher level of lipid peroxidation in aquatic organisms exposed to high concentrations of pollutants[5].

The primary causative agent identified in the pathogenesis of lead poisoning is lead induced oxidative stress.

Cite this article: Sharma, S., Vyas, V. and Tamot, S., Toxic effect of lead nitrate on lipid peroxidation and protein carbonyl content in the muscle of freshwater fish *Channa striatus* (Bloch, 1793), *Int. J. Pure App. Biosci.* **5(5):** 828-832 (2017). doi: http://dx.doi.org/10.18782/2320-7051.4055

Lead causes oxidative stress by stimulating the production of reactive oxygen species, lowering the antioxidant levels by reducing glutathione, obstructing with various metals and increasing vulnerability of cells by altering membrane integrity and fatty acid composition to oxidative attack[6]. To establish the variability of lipid peroxidation and protein carbonyl content in relation to lead nitrate exposure for varying periods, muscle from freshwater fish *C. striatus* were analyzed. The present study clearly indicates that lead nitrate alters oxidative stress parameters in the muscle of fish.

MATERIALS AND METHODS

Experimental Design

C. striatus of both genders (length 20-25 cm and weight 50-60 gm) were collected for experimental study from local fish markets of Bhopal, Madhya Pradesh (India). They were acclimatized to laboratory condition for a period of 15 days prior to the experiment. Fishes of experimental groups were divided into four groups having 15 fishes in each aquarium. Group I served as control and was maintained in normal water without any treatment while the fishes of groups 2 to 4 were treated with sub-lethal concentrations (8, 18 and 28 mg/l) of lead nitrate (Ranbaxy India Ltd.) for a period of 90 days. The LC_{50} values at 95% confidence limits for different exposure period were calculated by using the software "Trimmed Spearman Karber method", version-1.5[7]. The LC_{50} value under present experiment was observed to be 284.32 mg/l. To maintain desired lead nitrate concentration throughout the experimentation duration of 90 days, water of each aquarium was changed on every alternate day. At the end of 30^{th}, 60^{th} and 90^{th} days of experimentation, fishes from control and treated groups were dissected out for the removal of muscle, aseptically.

Estimation of lipid peroxidation

Homogenates of muscle were centrifuged at 3000 rpm for 15 min and the supernatant was used for lipid peroxidation. Lipid peroxidation was measured by the release of thiobarbituric acid reactive substance (TBARS) in terms of malondialdehyde equivalents (MDA) using a molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} as described by Ohkawa *et al*[8]. In this reaction, a colored complex was formed and absorbance was determined spectrophotometrically (UV/VIS spectrophotometer) at 532 nm. The lipid peroxidation level was expressed as nanomoles of thiobarbituric acid reactive substances formed per gram of tissue (nmol TBARS/g tissue).

Estimation of protein carbonyl content

Protein carbonyl groups were estimated by the method as described by Levine *et al*[9]. Homogenates of muscle were centrifuged at 4000 rpm for 10 min and the supernatant was used for protein carbonyl content. After centrifugation, the supernatant was divided equally into two tubes. 1.6 ml 2,4-dinitrophenylhydrazine (DNPH 10 mM) was added to precipitate in one tube and 1.6ml HCl (2M) is added to another tube and vortexed every 10-15 min for one hour. Proteins were precipitated with an equal volume of 20% TCA (trichloroacetic acid) and centrifuged at 4000 rpm for 10 min. The precipitated protein pellets of DNPH tubes were washed three times with 1.5 ml of ethyl acetate and ethanol (1:1). Final protein precipitates were dissolved in 2.5 ml NaOH (100 mM) and absorbance of both (DNPH and HCl tubes) at 370 nm was determined, spectrophotometrically. The values were expressed as nanomoles of protein carbonyl per gram protein (nmol/g protein).

Statistical Analysis

All data are expressed as mean ± standard deviation. The statistical evaluation of all data was done using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests using the computer software (Sigma Stat 3.5). P values <0.05 were regarded as statistically significant.

RESULTS AND DISCUSSION

Effects of lead nitrate on lipid peroxidation

Changes in lipid peroxidation in the muscle of fish after lead nitrate exposure at different concentrations (8, 18 and 28 mg/l) are shown

in Fig.1. Exposure of each concentration was extended for periods of 30, 60 and 90 days, which resulted in a significant increase ($p<0.05$) in lipid peroxidation in comparison to control. Lipid peroxidation of control fish was 1.001 ± 0.270 nmol TBARS/g tissue, 1.330 ± 0.203 nmol TBARS/g tissue and 1.618 ± 0.246 nmol TBARS/g tissue for a period of 30, 60 and 90 days, respectively. In the treated fishes at 8 mg/l lead nitrate concentration, lipid peroxidation was 2.888 ± 0.770 nmol TBARS/g tissue, 5.553 ± 2.345 nmol TBARS/g tissue and 7.959 ± 2.437 nmol TBARS/g tissue for a period of 30, 60 and 90 days, respectively. In the treated fishes at 18 mg/l lead nitrate concentration, lipid peroxidation was 3.840 ± 0.821 nmol TBARS/g tissue, 6.605 ± 2.018 nmol TBARS/g tissue and 10.528 ± 2.282 nmol TBARS/g tissue for a period of 30, 60 and 90 days, respectively. In the treated fishes at 28 mg/l lead nitrate concentration, lipid peroxidation was 6.692 ± 1.699 nmol TBARS/g tissue, 9.441 ± 2.349 nmol TBARS/g tissue and 21.192 ± 5.555 nmol TBARS/g tissue for a period of 30, 60 and 90 days, respectively.

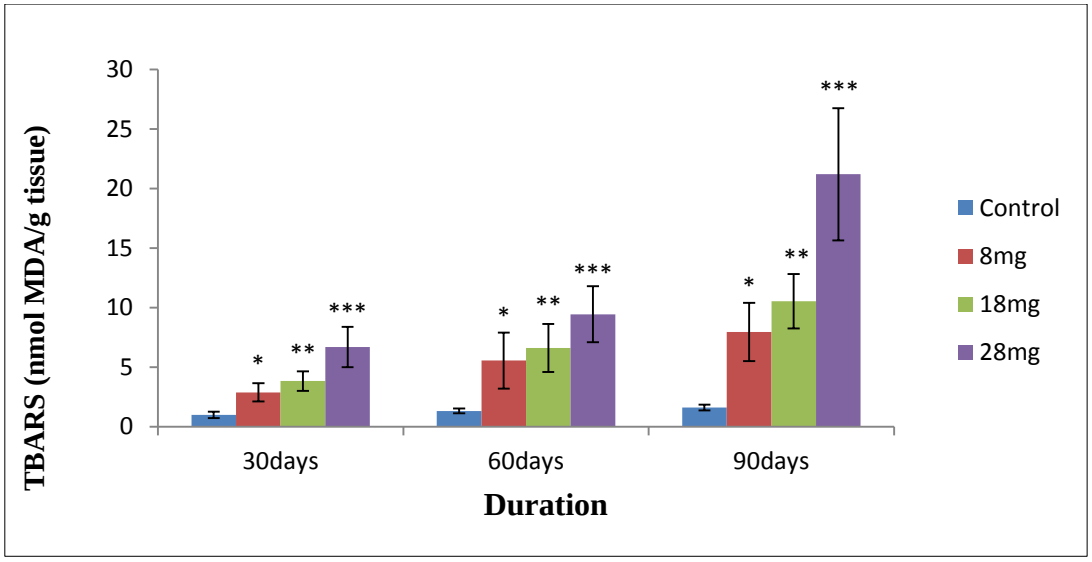

Fig. 1: Effect of Lead nitrate (8 mg/l, 18 mg/l and 28 mg/l) concentration on lipid peroxidation (nmole/g tissue) in the muscle of *C. striatus* for a period of 30, 60 and 90 days. *Indicates level of significance between control and treated groups (P<0.05)

Muscle is the main eatable part of fish and its bioanalysis is indispensable to monitor the quality of fish for human health and consumption[9]. As a result of high level of oxygen consumption, free radical generation is more pronounced in muscle[10]. As shown in Fig. 1, lipid peroxidation in the muscle of *Channa striatus* was increased significantly when compared with control. This increase is dependent on dose and duration of exposure. Similar findings were also observed by Fulle *et al.*[11] in muscle of hake *Merluccius merluccius*. Ognjanovic *et al.*[12] observed significant increase in the lipid peroxidation in white muscle of *Cyprinus carpio morpha* after exposure to copper sulphate[12] also reported increased level of lipid peroxidation in the muscle of *Heteropneustes fossilis*.

Effects of lead nitrate on protein carbonyl content

Changes in protein carbonyl content in the muscle of fish after lead nitrate exposure at different concentrations (8, 18 and 28 mg/l) are shown in Fig.2. Exposure of each concentration was extended for periods of 30, 60 and 90 days, which resulted in a significant increase ($p<0.05$) in protein carbonyl content in comparison to control. The protein carbonyl content of control fish was 13.565 ± 0.769 nmol/g protein, 13.565 ± 0.769 nmol/g protein and 14.765 ± 2.722 nmol/g protein for a period of 30, 60 and 90 days, respectively. In the

treated fishes at 8 mg/l lead nitrate concentration, protein carbonylation was 18.844 ± 5.088 nmol/g protein, 21.926 ± 6.386 nmol/g protein and 26.951 ± 4.955 nmol/g protein for a period of 30, 60 and 90 days, respectively. In the treated fishes at 18 mg/l lead nitrate concentration, protein carbonylation was 24.034 ± 3.294 nmol/g protein, 37.650 ± 4.362 nmol/g protein and 49.783 ± 4.059 nmol/g protein for a period of 30, 60 and 90 days, respectively. In the treated fishes at 28 mg/l lead nitrate concentration, protein carbonylation was 35.398 ± 4.805 nmol/g protein, 47.222 ± 4.699 nmol/g protein and 59.077 ± 6.986 nmol/g protein for a period of 30, 60 and 90 days, respectively.

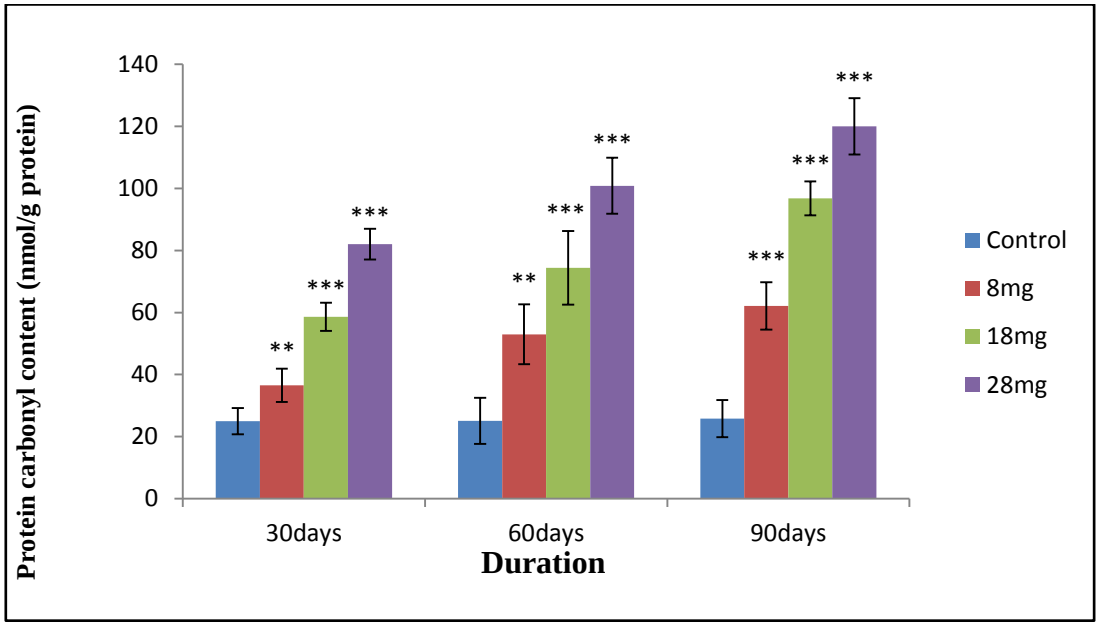

Fig. 2: Effect of Lead nitrate (8 mg/l, 18 mg/l and 28 mg/l) concentration on protein carbonyl content (nmole/g protein) in the muscle of *C. striatus* for a period of 30, 60 and 90 days. *Indicates level of significance between control and treated groups (P<0.05)

Muscles functions basically by active cooperation between various proteins and their movements are very much required for swimming and other various activities of fishes. As shown in Fig. 2, protein carbonyl content in the muscle of *Channa striatus* were increased significantly when compared with control. Similar findings were also observed by Borthakur[14]. Parvez and Raisuddin[15] reported a significant increase in protein carbonylation level in white muscle of Nile tilapia (*Oreochromis niloticus*) after chronic exposure to total ammonia nitrogen (TAN) at 5 mg/l and 10 mg/l concentration for 70 days.

From the present study it is concluded that lead nitrate causes adverse effect in the muscle of fish with respect to changes in lipid peroxidation and protein carbonyl content.

Acknowledgement

The authors are thankful to Head, Department of Zoology and Applied Aquaculture, Barkatullah University, Bhopal (M.P) India, for providing necessary laboratory facilities to carry out the present work successfully.

REFERENCES

1. Ferreira, M., Moradas-Ferreira, P., and Reis-Henriques, M. A. Oxidative stress biomarkers in two resident species, mullet (*Mugil cephalus*) and flounder (*Platichthys flesus*), from a polluted site in River Douro Estuary, Portugal. *Aquatic Toxicology.* **71(1):** 39-48 (2005).
2. Thirumavalan, R. Effect of mercuric chloride on lipid peroxidation and glutathione in the fresh water fish, *Catla catla*. *International Journal of Modern*

Research and Reviews. **2(3):** 129-131(2014).

3. Kumar, M., Trivedi, S. P., Misra, A., and Sharma, S. Histopathological changes in testis of the freshwater fish, *Heteropneustes fossilis* (Bloch) exposed to linear alkyl benzene sulphonate (LAS). *Journal of Environmental Biology*. **28(3):** 679-684 (2007).
4. Livingstone, D. R. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. *Marine Pollution Bulletin*. **42(8):** 656-666 (2001).
5. Viarengo, A. Heavy metals in marine invertebrates, mechanisms of regulation and toxicity at cellular concentrations. *Review in Aquatic Science*.**1:** 295-317 (1989).
6. Sharma, S., Sharma, V., Pracheta, and Sharma, S. H. Therapeutic potential of hydromethanolic root extract of *Withania somnifera* on neurological parameters in Swiss albino mice subjected to Lead nitrate. *International Journal of Current Pharmaceutical Research*. **3(2):** 52-56 (2011).
7. Hamilton, M. A., Russo, R. C., and Thurston, R. V. Trimmed Spearman-Karber method for estimating median lethal concentration in toxicity bioassays. *Environmental Science and Technology*. **11(7):** 714-719 (1977).
8. Ohkawa, H., Ohishi, N. and Yagi, K. Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. *Analytical Biochemistry*. **95(2):** 351-358 (1979).
9. Levine, R. L., Garland, D., Oliver, C. N., Amici, A., Climent, I., Lenz, A. G., Ahn, B. W., Shaltiel, S. and Stadtman, E. R. Determination of carbonyl content in oxidatively modified protein. *Methods in Enzymology*. **186:** 464-478 (1990).
10. Begum, G. and Vijayaraghavans, S. Effect of acute exposure of the organophosphate insecticide roger on same biochemical aspects of *Clarias batrachus* (Linnaeus). *Environmental Research*. **80(1):** 80-83 (1999).
11. Fulle, S., Protasi, F., Di Tano, G., Pietrangelo, T., Beltramin, A., Boncompagni, S., Vecchiet, L. and Fango, G. The contribution of reactive oxygen species to sarcopenia and muscle ageing. *Experimental Gerontology*. **39(1):** 17-24 (2004).
12. Ognjanovic, B. I., Milovanovic, J. G., Dordevic, N. Z., Markovic, S. D., Zikic, R. V., Stajn, A. S. And Saicic, Z. S. Parameters of oxidative stress in liver and white muscle of hake (*Merluccius merluccius* L.) from the Adriatic Sea. *Kragujevac Journal of Science*. **30:** 137-144 (2008).
13. Radi, A. A. and Matkovics, B. Effects of metal ions on the antioxidant enzyme activities, protein contents and lipid peroxidation of Carp tissues. *Comparative Biochemistry and Physiology* C: *Comparative Pharmacology*. **90(1):** 69-72 (1988).
14. Borthakur, M. Kr. Comparative study of tissue lipid and protein peroxides as oxidative stress markers in sublethal malathion exposed Indian catfish *Heteropneustes fossilis. International Journal of Pure and Applied Bioscience*. **3(2):** 407-412 (2015).
15. Parvez, S. and Raisuddin, S. Protein carbonyls: novel biomarkers of exposure to oxidative stress-inducing pesticides in freshwater fish *Channa punctata* (Bloch). *Environmental Toxicology and Pharmacology*. **20 (1):** 112-117 (2005).
16. Hegazi, M. M., Attia, Z. I. and Ashour, O. A. Oxidative stress and antioxidant enzymes in liver and white muscle of Nile tilapia juveniles in chronic ammonia exposure. *Aquatic Toxicology*. **99 (2):** 118-125 (2010).