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Lead nitrate induced biochemical change on gonad and gonadal development of Catfish (*Clarias batrachus* (L.)

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ABSTRACT

The present paper deals with the effect of Lead nitrate on the gonads and the gonadal development of common Indian catfish, *Clarias batrachus* (L.). It has been observed that the Gonadosomatic Index of both male and female were reduced significantly in case of lead treated fish. A great increase of primary (non-yolky) oocytes was observed in treated ovary. Mature oocytes were totally disappeared and atretic follicles were found. Whereas, control ovary had fully mature vitellogenic stage IV oocytes. The lead treated testis was completely devoid of spermatids and mature sperms. Cells of vas deferens were crumpled often with inter-cellular spaces having irregular nuclei. All the cross sections of control testis were occupied by the sperms that filled several tubules. Summarily, it can be concluded that the Lead nitrate has severe adverse impacts on gonadal development showing biochemical alterations.

1. Introduction

Lead is potent toxic metal, the quantity of which is progressively increasing in our environment (Choudhury and Mudipalli 2008; Directive 2000; Bhakta et al. 2012; Bhakta and Munekage 2012). Most of the lead salts find their way into aquatic systems through rain, sewages and effluents. Absorption of lead by aquatic animals is affected by the age, gender and diet of the organism, as well as the particle size, chemical species of lead and presence of other compounds in the water. Lead is known to affect the structure and function of various organs and tissues. It is highly toxic to aquatic organisms, especially fish. Fish from polluted areas do build up substantial concentrations of lead in muscle tissue and whole-body analysis of fish for lead is still recommended for general environmental monitoring. The biological effects in sublethal concentrations of lead include delayed embryonic development, suppressed reproduction, inhibition of growth, increased mucous formation, neurological problems, enzyme inhibition and kidney dysfunction. Sublethal effects of lead on pituitary function of rainbow trout during exogenous vitellogenesis were studied by Ruby et al. (2000). Alados and Daniel (1999) reported the effect of lead on the predictability of reproductive behavior in fathead minnows, *Pimephales promelas.* The fish, *Clarias batrachus* is a hardy fish and highly valued in India specially West Bengal. It would therefore be of interest to study the quantity of lead that can be accumulated within its tissues. So, this work is therefore aimed to assess the toxic effect of lead on the ovary and testis of *Clarias batrachus* (L.).

2. Materials and methods

About 80 catfishes, *Clarias batrachus* measuring 20 – 22 cm and weighing 90 – 100 g were brought from local market and were acclimatized to laboratory conditions for about 2 weeks prior starting the experiment. They were kept in tap water. The fishes were divided into four groups of 20 each. Three groups were exposed continuously to 3.0, 5.0 and 7.0 ppm lead nitrate for 150 days and the fourth group was given no treatment and served as control. The water temperature varied from 20 – 35°C from February to June. They were fed

with minced goat liver and the water was changed every alternate day after feeding the fish. At the end of the experiment, the fish were weighed and sacrificed, gonads were dissected out within 1 – 2 min, rapidly frozen and processed for various biochemical analysis. The significance of analysis was ratified through student's t-test. For histological preparation, ovary and testis from both the experimental and control groups were fixed in Bouin's fixative for 24 hours. After that, paraffin blocks containing the tissue were sectioned at 6 µm thickness and then stained by double staining (Haematoxylene and Eosin) method.

3. Results

The fish, *Clarias batrachus* is an annual breeder and it spawns during the monsoon months starting from late June to early September. The treatment of lead nitrate was given in February when the gonads were in resting phase and terminates in June when they were fully matured in the controls. Histology of treated fish was largely made up of stage-I oocytes. However, few stage II oocytes and some larger oocytes were also encountered (Plate 1a). In some cases larger oocytes showed signs of atresia. The control ovary had fully mature vitellogenic stage IV oocytes (Plate 1b). The testis of treated fish had only spermatogonial cells and spermatocytes whereas, in the control, the tubular lumen was filled with sperm. Comparatively from 3.0 ppm, in the 5.0 ppm lead exposed fish, the colour becomes darker which is more pronounced in the tail. Marginal erosion and extravasation of blood in the tail region of some was noticed after 90 days of treatment. When exposed to7.0 ppm such changes were obvious after 40 days. Table 1 shows the significant decreases of primary oocytes, GSI and ova diameter in the lead treated fish when compared to that of controls.

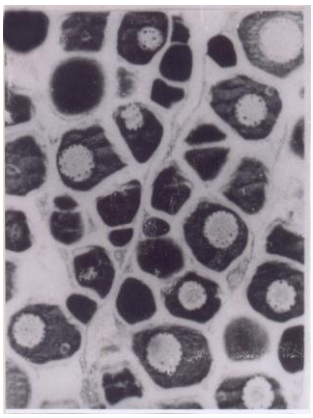
Histological examination of the testis following chronic exposure of the fish to lead nitrate showed a very significant decrease of the testicular diameter. The lead treated testis was completely devoid of sparmatids and spermatozoa. The area occupied by the spermatids and sperms were largely filled with amorphous masses containing dark stained nuclei and few irregular cells (Plate 2a). In the lead treated fish, living cells of vas deferens were crumpled often with intercellular spaces having irregular nuclei. The blood supply appeared more conspicuous than controls and the process of spermiogenesis was affected. Whereas, in the control fish the testis is divided into lobes and lobules separated by intralobular stromal fibres and several nest of pre spermiogenic germ cells and vast masses of spermatids and sperms were noticed. In fact, a significant area of the cross sections of the testis was occupied by the sperms that filled several tubules (Plate 2b).

Table 1. Effect of lead nitrate on the ovary of *Clrias batrachus.* Figures represent mean ± S.D

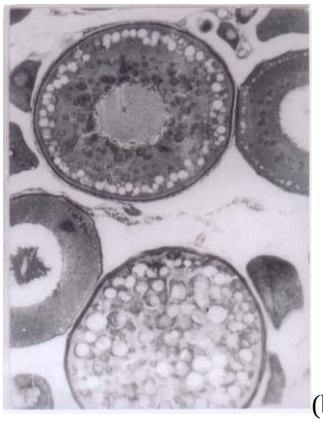
Parameters	**Control**	**Treated**
Gonadosomatic index	6.73 ±0.72 [a]	2.17 ±0.01 [b]
Ovary diameter(um)	8212.00 ±188.20 [a]	1871.01±137.70 [b]
Oogonia	15.00 ± 1.08 [a]	21.00 ± 0.80 [b]
Primary (non-yolky) oocytes	19.00 ±0.64 [a]	46.00 ± 2.16 [b]
Primary(yolky) oocytes	32.00 ±078 [a]	2.00 ± 0.04 [b]
Mature oocytes	41.00 ± 1.40 [a]	Absent
Atretic follicles	2.00 ± 0.25 [a]	34.00 ± 4.21 [b]

[The different superscripts in a row are significantly different at p≤0.05] Each data is a mean of five separate determinations.

Plate 1 a) treated ovary and b) control ovary



(a)



(b)

Plate 2 a) treated testis and b) control testis


(a)


(b)

Table 2. Effect of lead nitrate on the testis of *Clarias batrachus*. Figures represent mean ± S.D.

Parameters	Control	Treated
Gonadosomatic index	0.29 ± 0.01 [a]	0.12 ± 0.02 [b]
Testicular diameter (µm)	5789.25 ± 298.60 [a]	1369.40 ± 140.25[b]
Primary spermatogonia	19.00 ± 0.56 [a]	28.00 ± 1.65 [b]
Secondary spermatogonia	22.00 ± 1.10 [a]	35.00 ± 0.85 [b]
Primary spermatocytes	32.00 ± 1.45 [a]	71.00 ± 1.39 [b]
Secondary spematocytes	77.00 ± 1.00 [a]	13.00 ± 0.23 [b]
Spermatids	90.00 ± 1.00 [a]	Absent
Spermatozoa	448.00 ± 2.00 [a]	Absent

[The different superscripts in a row are significantly different at $p \leq 0.05$]. Each data is a mean of five separate determinations.

The data recorded in Table 1 and 2 showed the mean value of variation in GSI, gonadal diameter, differential count and cytomorphology of germinal cells in the ovary and testis of both lead treated and control fish which are statistically quite significant. Gonadal development and maturation are intimately correlated with GSI. In present investigation, GSI of control groups were higher than treated groups and in case of treated groups gonadal development was also affected. So, it showed that the reduction of GSI was directly correlated with gonad maturity.

Upto 90 days of experiment, the mortality rate was nil. After 150 days, in case of both the control and treated fishes with first dose of Lead Nitrate (3.0 ppm) there was no mortality observed (Fig 1). But fishes with next two doses that were 5.0ppm and 7.0 ppm, there observed 2% and 6% mortality respectively due to prolong treatment of Lead Nitrate (Table 3).

Table 3. The mortality percentage after 150 days of both control and treated fish

Group	Doses	Mortality %
Control		NIL
Treated	3 ppm	NIL
	5 ppm	2%
	7 ppm	6%

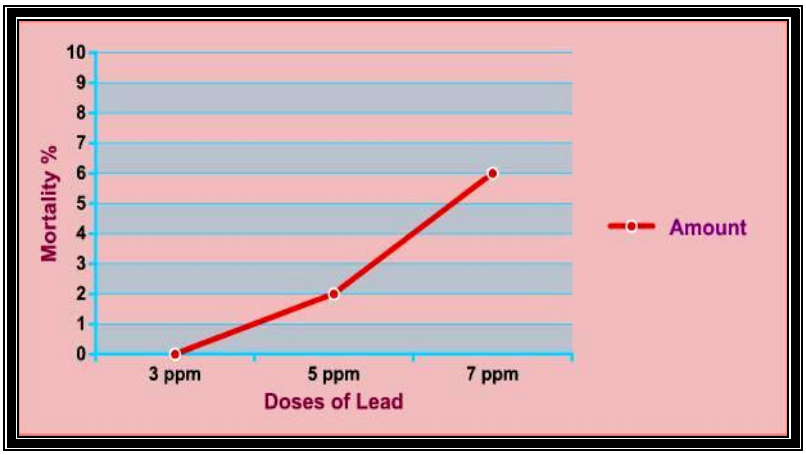


Fig 1 Mortality percentage of *Clarias batrachus* (L.) in different doses of Lead nitrate treatment

4. Discussion

All measured effects of lead on living organisms are adverse, including those related to survival, growth, learning, reproduction, development, behavior and metabolism. Lead interferes with biochemical, physiological, morphological and behavioral parameters of fish. Lead functions as a cumulative poison. Lead is a heavy metal which is very toxic to aquatic organisms, especially fish. Various experiments were conducted about the toxicity of heavy metals with fresh water fish but little work has been done about the effects of Lead (Pb) in the sub lethal concentration on the gonadal development of fresh water fish. As reported in mammals including man (Vallee and Ulmar 1972) and in a few fish, changes in the activities of some enzymes were reported in response to exposure to sub lethal level of Lead. According to Tulasi et al. (1989) accumulation studies indicated that lead accumulated in the brain of *Anabas testudineus*, probably changing specific gonadal functions, resulting in an altered reproductive potential. They also found that the exposure of lead hastened the spawning of *A. testudineus*, possibly due to elevated corticosteriod levels. James et al. (1996) reported the effects of lead on respiratory enzyme activity, glycogen and blood sugar levels of the teleost, *Oreochromis mossambicus* (Peters) during accumulation and depuration. Olaifa et al. (2003) studied the toxic effect of lead on *Clarias gariepinus* (African catfish) fingerlings.

The toxic stress of lead on fish was tested employing a 96-hour bioassay test. The experimental fish used were *Clarias gariepinus* fingerlings. Lead in the form of lead chloride was used to prepare the stock solution. The concentrations of lead used for the experiment were 0, 1.8, 3.2, and 5.6, and 10.0mgl^{-1}. Kasthuri and Chandran (1997) studied the sublethal effect of Lead on feeding energetics, growth performance, biochemical composition and accumulation of the estuarine catfish, *Mystus gulio* (Hamilton). Lead has to decreasing trend in feeding energetics, growth rate and biochemical composition when compared to their respective controls. Of the three size groups, fingerlings seemed to be more sensitive to lead poisoning followed by the immature and then by the mature fish.

In the recent investigation with *Clarias batrachus*, Lead caused degenerative changes in the both gonads. In the control ovary, matured vitellogenic Stage IV oocytes were found whereas, in case of treated fish mainly Stage I and few Stage II oocytes were encountered. The testis of treated fish had only spermatogonial cells and spermatocytes but in the control fish, mature sperms were seen. Thus the present study reveals that long term exposure to lead caused retardation of gonadal growth, reducing gonadosomatic index which was directly related with reduced gonad weight and reduction of mature oocytes and sperms.

4. Conclusion

The present study revealed the severe adverse impacts of Lead nitrate on gonadal development of *Clarias batrachus* along with biochemical alterations.

References

Alados CL, Daniel NW (1999) Lead effects on the predictability of reproductive behavior in fathead minnows (*Pimephales promelas*). Environmental Toxicology and Chemistry. 18 (10) **:** 2392–2399.

Bhakta JN, Munekage Y, Ohnishi K, Jana BB (2012) Isolation and identification of cadmium and lead resistant lactic acid bacteria for applying as metal removing probiotic. International Journal of Environmental Science and Technology 9:433–440.

Bhakta JN, Ohnishi K, Munekage Y, Iwasaki K, Wei M (2012) Characterization of lactic acid bacteria-based probiotics as heavy metals sorbents. Journal of Applied Microbiology 112:1193–1206.

Choudhury H, Mudipalli A (2008) Potential considerations andconcerns in the risk characterization for the interaction profiles of metals. Indian J Med Res 128:462–483.

Directive 2000/60/EC (2000) Water Framework Directive of theEuropean Parliament and of the Council of 23 Oct 2000

James R, Sampath K and Alagrathinam S (1996) Effects of lead on respiratory enzyme activity, glycogen and blood sugar levels of the teleost, *Oreochromis mossambicus* (Peters) during accumulation and depuration. Asian Fish. Sc. 9(2): 87–100.

Kasthuri J and Chandran MR (1997) : Sublethal effect of lead on feeding energetics, growth performance, biochemical composition and accumulation of the estuarine catfish, *Mystus gulio* (Hamilton). J Environ Bio, 18(1): 95–101.

Olaifa FE, Olaifa AK, Lewis OO (2003) Toxic stress of Lead on *Clarias gariepinus* (African catfish) fingerlings. African Journal of Biomedical Research 6(2).

Roberts JR (1999) Metal toxicity in children. In: training manual on pediatric environmental health: putting it into practice. Emeryville, CA, Children's Environmental Health Network. http://www.cehn.org/cehn/training manual/(pdf/manual-full.pdf).

Ruby SM, Jaroslawski P, Hull R (1993) Lead and cyanide toxicity in sexually maturing rainbow trout, *Oncorhynchus mykiss* during spermatogenesis. Aquat. Toxicol. 26(3-4): 225–238.

Tulasi SJ, Reddy PUM, Ramano Rao J.V (1989) Effects of lead on the spawning potential of the fresh water Fish, *Anabas testudineus*. Bull. Environ.Contam. Toxicol. 43: 8–863.

Vallee BL, Ulmer DD (1972) Biochemical effects of mercury, cadmium and lead. Ann. Rev. Biochem 4: 91–128.