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Research Paper

Histopathological changes in nucleus preopticus of *Heteropneustes fossilis* exposed to lead acetate and its amelioration with *Ocimum sanctum* and *Curcuma longa*

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Abstract

The present study was carried out to evaluate the toxic effect of lead acetate (5ppm) on NPO in stinging catfish, *Heteropneustes fossilis* for a period of 21 days under laboratory conditions. The most common resultant changes in NPO of fish to lead acetate were vacuolization, clumping of cytoplasm, loss of staining affinity, hypertrophy, reduced neurosecretory material and degeneration as compared to control group. The fishes exposed to lead acetate along with tulsi and turmeric showed recovery in their nuclei, reduced hypertrophy and vacuolization, indicating their protective effects against Pb toxicity.

Keywords: Lead, Nucleus Preopticus, *Ocimum sanctum*, *Curcuma longa, H. fossilis*.

Introduction

The pollution caused by heavy metals might have dreadful effects on the ecological equilibrium and a variety of aquatic entities[1,2]. Heavy metal contamination of aquatic environment has drawn increasing attention as it may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms. These metals tend to accumulate in organisms and have been found to have a variety of adverse effects on fishes. The higher concentrations of lead, cadmium, mercury etc. were toxic to fishes[3,19]. Dangerous pollutant that can be absorbed by fish when exposed to elevated levels in an aquatic environment is Lead (Pb). The absorption of lead occurs by different ways through gills and skin or by ingestion of contaminated water and food; and may lead to high mortality rate or cause many biochemical and histological alterations in survived fish[7]. Most of this lead (Pb) is used for batteries and the remainder is used for cable coverings, plumbing, fuel additives, paint pigments, PVC plastics, X-ray shielding, crystal glass production, mining, smelting, refining, gasoline, ceramic glazing and the making of stained glasses[1].

The hypothalamus in vertebrate brain comprises groups of neurosecretory cells which control secretion of the various tropic hormones of the pituitary by elaborating releasing (-RH) and inhibiting hormones (-IH) reported by Perer et al. Hypothalamo-neurosecretory system comprises mainly of Gomori-positive Nucleus Preopticus (NPO) and the Gomori-negative Nucleus Lateralis Tuberis (NLT) and their axonal tracts[12-13]. The NPO are paired structure and situated in the walls of the hypothalamus on either side of the third ventricle and anterodorsal to the optic chiasma. The limb of the NPO lies horizontally to the pituitary stalk. The other limb of the NPO is ventricle to the optic nerve and forms a wide plate of the cells. The ventral half of this plate is made up of smaller cells known as pars parvocellularis (PPC) and other horizontal limb forms pars magnocellularis (PMC) consisting of large neurosecretory neuronal cells at the dorsal half of the plate. Thus progressive reductions in the

size of neurosecretory cells have seen from the dorsal to the ventral aspect of NPO[9]. There exist reports that NPO is involved in the spawning activities and its secretion does influence gonadal maturation among teleosts[14].

Tulsi (*Ocimum sanctum*) and Turmeric (*Curcuma longa*) have also been shown to protect against the toxic effects of heavy metals such as lead, arsenic, cadmium, chromium and mercury[5,15]. Tulsi contains different minerals including calcium, phosphorus, carotene, iron, zinc and vitamin contents like vitamin A, vitamin C, vitamin E, which binds to the heavy metals and carry them out of the body[11,21]. Curcumin is the main curcuminoid of turmeric. The presence of curcuminoids, proteins and carbohydrates, turmeric powder provides a variety of binding sites for metal ions and act as a natural chelate[16,22]. Hence, the present work was undertaken to study the dose dependent impact of lead acetate on histological changes in the structure of the Nucleus Preopticus of *H. fossilis* and also investigate the role of *Ocimum sanctum* and *Curcuma longa* on lead induced toxicity.

Materials and methods

Fish collection: Live, healthy specimen of *Heteropneustes fossilis* measuring about 12±20 cm and weighing 9±15gm were collected for experimental study from local fish market of Ujjain and used as the test animals.

Metal used: Lead was used for present experiment in the form of lead acetate [$Pb(CH_3COO)_2$]. Stock solution of lead acetate was prepared by taking computed amount (2g) and made up to the desired volume (1L) using distilled water to achieve a stock of 2g/L. The dose of lead acetate was decided by LC_{50} value. The safe dose given to fishes was kept at 5ppm.

Recovery agent: *Ocimum sanctum* and *Curcuma longa* were used as recovery agents in the present investigation.

Experimental fish: Before introducing in the aquarium, fishes were treated with 0.01% $KMnO_4$ solution to avoid dermal infection. The fishes were quarantined and acclimatized in laboratory aquarium for 15 days prior to the start of experiment. All the fishes of experimental groups were fed with dried and chopped prawns twice a day. Aquarium water was renewed on every alternate day and aerated by an aquarium pump for 30 minutes daily. The lead acetate was maintained throughout the experimental duration of 21 days.

Experimental design: The details of experimental groups are given in the table:

Table 1: Dose and food given to *Heteropneustes fossilis* in control, treated and recovery group

S. NO.	Groups	Treatment
1	I (Control group)	Plain food (without treatment)
2	II(Experimental group)	Exposed to 5ppm of lead acetate + Plain food.
3	III (Recovery group I)	Exposed to 5ppm of lead acetate + Food with *Ocimum sanctum* (Tulsi)
4	IV (Recovery group II)	Exposed to 5ppm of lead acetate + Food with Curcuma longa (Turmeric)

The fishes were kept for 21 days from the date of commencement of the experiment.

Histopathological procedure: On the 7th, 14th and 21^{lst} day of exposure, fish from control, treated and recovery group were sacrificed. The pituitary with entire brain was removed and fixed in aqueous Bouin's fixative for 24hrs. Preserved tissues were washed under tap water, dehydrated in ascending grades of ethanol, cleared in xylene and embedded in paraffin blocks. The paraffin blocks were then cut to 5µ (micron) thickness using rotatory microtome and stretched on clean glass slides. All the sections were stained routinely with CAHP (Chrome Alum Hematoxylin Phloxine)[4] after deparaffinization, mount in DPX and were examined under light microscope for histopathological examination. All the data and results for final observation were expressed in the form of microphotographs.

Results and Discussion

On histological examination, results indicated that the hypothalamo-hypophysial complex of *H. fossilis* consisted mainly of nucleus preopticus (NPO), nucleus lateralis tuberis (NLT), a complicated network of neurosecretory tracts and neurohypophysis which largely arborises in the pars intermedia. The NPO are paired structure situated on either side of the third ventricle dorsal to the optic chiasma. These cells were spherical or varied in shape from oval to round with evenly distributed cytoplasm and a single nucleus which is generally round in shape. The perikarya of neurons of NPO were with adequate quantity of neurosecretory material (Figure 1). Thus NPO is highly vascularized structure and its neurosecretory cells were positive to chrome alum hematoxylin phloxine (CAHP).

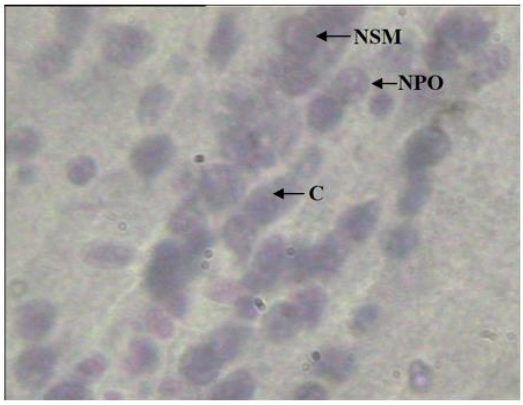


Figure 1: Control group: Showing NPO cells with bright stain and in normal structure.

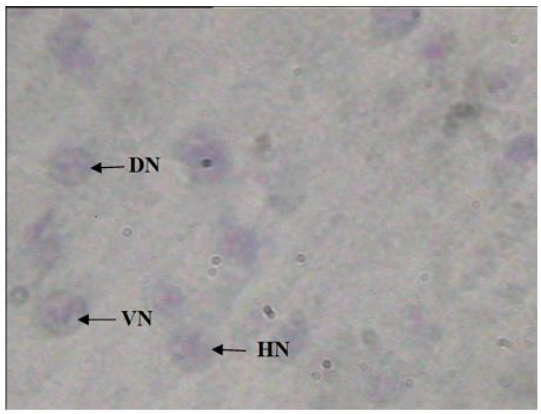


Figure 2: Treated group (7 days): Depicting cytoplasmic vacuolization, disruption and atrophic condition of NPO neurons

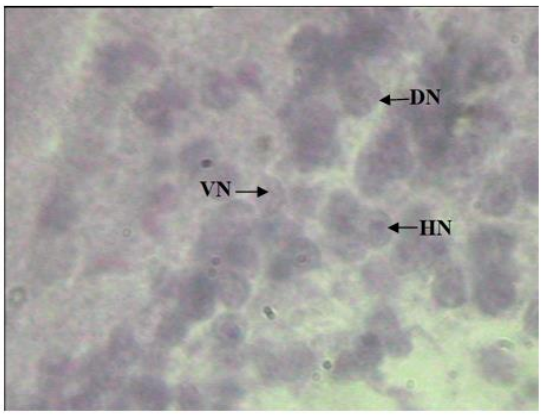


Figure 3: Treated group (14 days): Exhibiting deformed NPO cell bodies, partial loss of staining and hypertrophied neurosecretory cells

The present study documents histological changes in NPO cells of *H. fossilis* treated with lead acetate. During 7 days of exposure, most of the cells were smaller in size with depleted neurosecretory material in perikarya. The NPO neurons exhibited thick cell boundaries, enhanced

vascularization, turgid nuclei and partial loss of staining affinity and clumping of cytoplasm (Figure 2). The NPO neurons at 5ppm of lead acetate for 14 days duration exhibited cytoplasmic and nuclear abnormalities. These neurosecretory cells showed cytoplasmic degeneration and their cell boundaries were disappeared. These NPO cells became denatured and also lost their staining affinity (Figure 3).

More drastic changes were seen during the 21 days of lead acetate exposure, depicted exhausted condition due to lead (Pb) stress. These cells disappeared at places due to loss of their cellular contents and only coarse degenerated mass were exhibited. Their some nuclei showed lysis, devoid of cytoplasm and some cells lost their normal shape. They had little neurosecretory material and their cytoplasm and nuclei became vacuolated. Also, in 21 days treated group these cells exhibited faintly stain with CAHP (Figure 4).

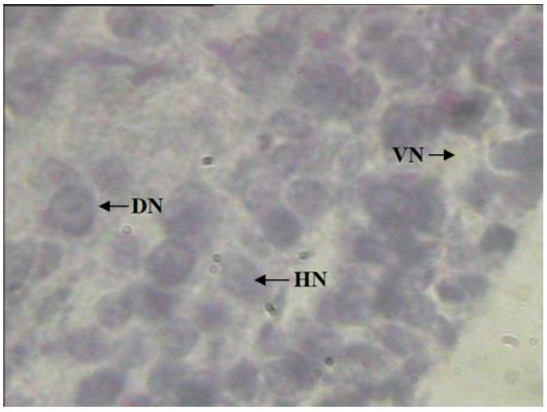
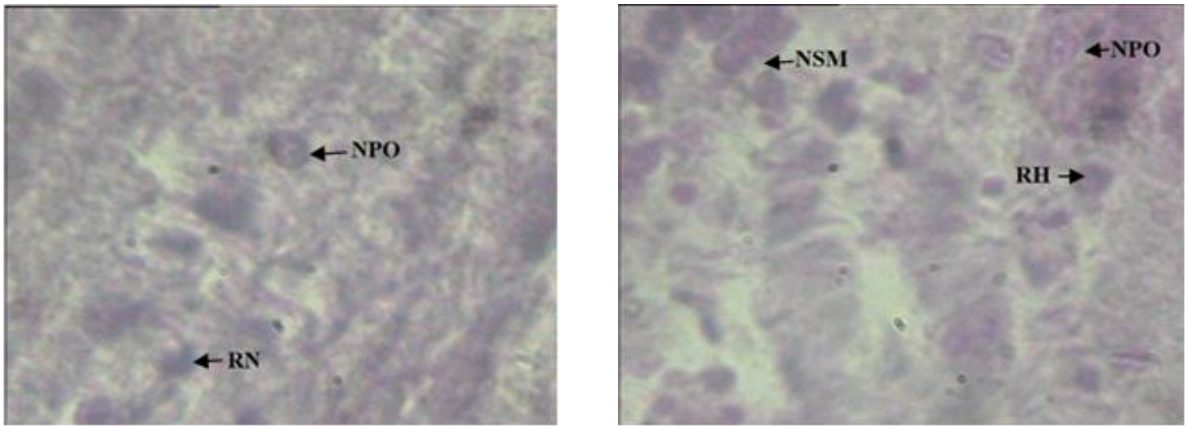


Figure 4: Treated group (21 days): Showing excessive vacuolization, hypertrophied nature, cytoplasmic disruption and less staining affinity of NPO neurons. Microphotographs of the L. S. of hypothalamus showing NPO of *Heteropneustes fossilis* in control and treated group (7, 14, 21 days: CAHP X1000).

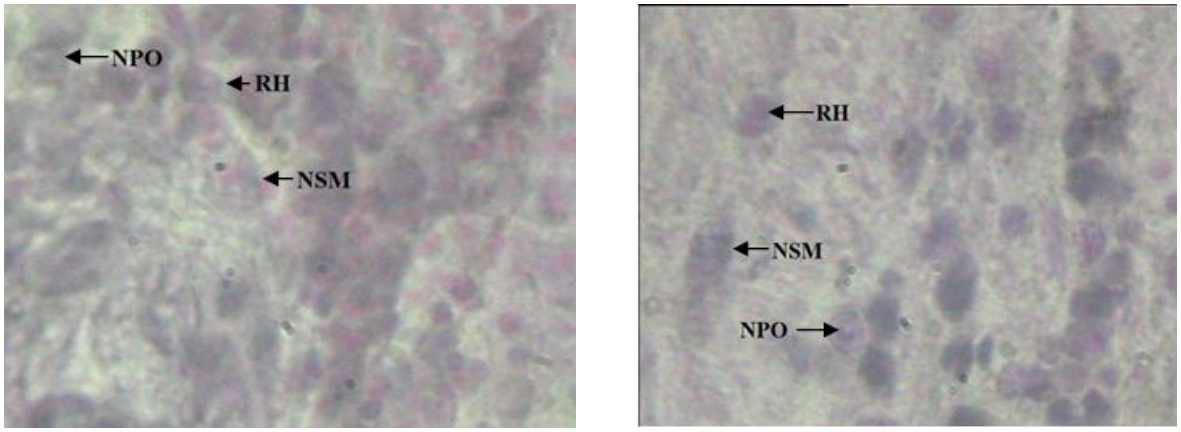
The histopathological findings mentioned were similar to those reported by Pandit and Bhattacharya[10]. They observed that the exposure of mercury chloride (0.01ppm) for 21 days to *H. fossilis* showed exhausted condition, reduced neurosecretory material, vacuolization, necrosis, degeneration and cytoplasmic clumping in these NPO neurons. They became hypertrophied, denatured and lost their staining affinity. Shukla and Pandey[20] observed almost similar responses in NPO neurons after exposure of 0.01ppm sublethal concentration of Endosulphan for 20 days in *Sarotherodon mossambicus*. Ram et al., stating that chronic exposure of carbofuran (4.5ppm) for six months to *Channa punctatus* showed darkly stained necrotic neurons, inactivity in neuron cells and loss of neurosecretory material in the NPO[18]. The NPO neurons exhibited thick cell boundaries, clumping of cytoplasm, depletion of neurosecretory material and hypertrophy in the teleost, *H. fossilis* in response to 0.5ppm exposure of cadmium chloride for 21 days[8]. Katti and Sathyanesan[6] in *Clarias batrachus*, Ram and Joy[17] observed that the exposure of sublethal concentrations of lead and mercuric chloride *to Channa punctatus* showed varying degrees of inactivity and degenerative changes in the neurosecretory cells of hypothalamus.

The present results have clearly demonstrated the ability that when lead acetate pretreated fish fed on tulsi and turmeric separately, showed recovery responses in damaged NPO neurosecretory cells. The NPO of tulsi recovery group depicted reduced cytoplasmic vacuolization due to homogenously distributed cytoplasm. The nuclei in these neurons were distinctly developed and stained normally with CAHP stain. Most of these cells recouped structurally (Figure 5(A), 6(A), 7(A). Whileas in turmeric recovery group of the same period, hypertrophied condition was gradually reduced and their cellular contents exhibited with reduced vacuolization. These cells depicted increased population with some extent of neurosecretory material and showed strong staining affinity (Figure 5(B), 6(B), 7(B). 10). These results of our study agreed well with the findings of Mukati et al.,[8] stating that the Ashawagandha showed signs of reconstitution in the neurons, regenerated cytoplasm, reduced hypertrophy and increased population with some extent of neurosecretory material against cadmium chloride (0.5ppm) for 21 days of exposure to *H. fossilis*.



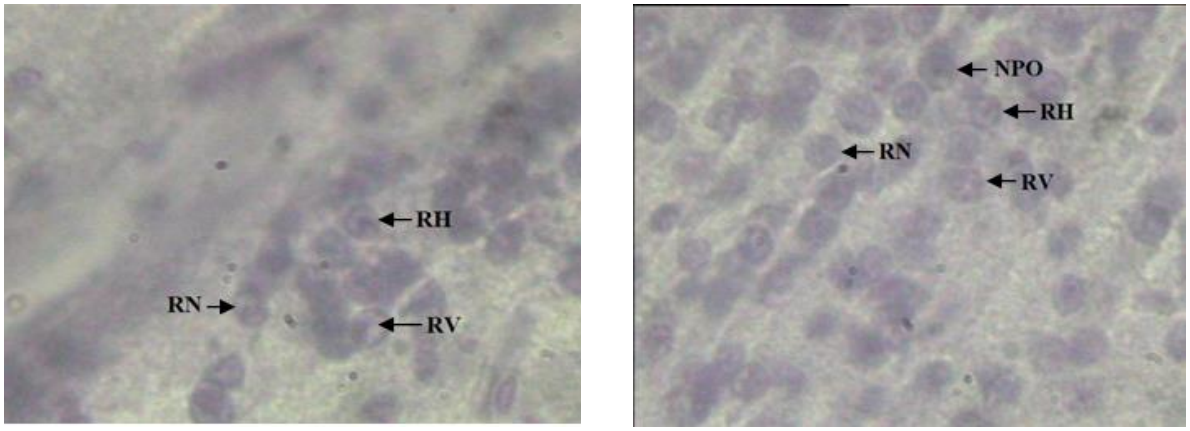
(A) **(B)**

Figure 5: (A) Tulsi and (B) Turmeric recovery group (7 days): Depicting reformed condition, reduced cytoplasmic vacuolization and distinctly developed nuclei in NPO cells



(A) (B)

Figure 6: (A) Tulsi and (B) Turmeric recovery group (14 days): Showing reduced hypertrophy, regeneration of neurosecretory material and reformed shape of NPO neurons



(A) (B)

Figure 7: (A) Tulsi and (B) Turmeric recovery group (21 days): Exhibiting regenerated cytoplasm, increased neurosecretory material and reconstitution of NPO neurons. Microphotographs of the L.S. of hypothalamus showing NPO of *Heteropneustes fossilis* in recovery groups (7, 14, 21 days: CAHP X1000).

Abbreviations:

NPO	Nucleus preopticus	C	Cytoplasm	DN	Degenerated neurons
HN	Hypertrophied neurons	VN	Vacuolized neurons	RV	Reduced vacuolization
NSM	Neurosecretory material	RN	Regenerated neurons	RH	Reduced hypertrophy

Conclusion

The histological changes show direct response of the animals to the pollutants and can be used as a sensitive model to monitor the aquatic pollution. The current study evidenced that lead is highly toxic and had a detrimental impact on the pituitary NPO responses of the fish, *H. fossilis* at 5ppm of lead acetate concentration. The destruction of pituitary NPO by lead affects the release of gonadotropic hormone that regulates gonadal function via changes in hypothalamo-hypophysial-gonadal axis and also affects the central peripheral nervous system. The herbal compounds tulsi and turmeric were almost found effective in controlling lead induced changes in NPO. However, further removal of other heavy metals can also be tried for further research work on the basis of the anti-toxic mechanism of tulsi and turmeric in experimental animals.

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