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Lead induced toxicopathic corneal lesions in *Ctenopharyngodon idellus* (Cuvier and Valenciennes)

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Abstract

Toxicological studies of lead nitrate on cornea of the fish, grass carp was investigated. 96 h LC_{50} of lead nitrate to *Ctenopharyngodon idellus* was determined by Probit analysis and it was found to be 33.27 mg/l. Toxicopathic corneal lesions were assessed by histopathology and scanning electron microscopy by exposing fish to sublethal concentration of lead nitrate- 11.09 mg/l (1/5th of LC50) for 15, 30 and 45 days. Histopathological alterations were characterized by sloughing off of the epithelial layer, degeneration of the fibrillar mat of stromal layer, erosion and heavy necrosis of epithelial cells. In addition, wrinkled and shrinked central region, uplifted epithelial layer, appearance of globular structures, detached irregular cells, severely damaged apical region and shrinked microridges were found under scanning electron microscopic studies. The results indicate that lead exposure has adversely affected the vision of the fish, attributable to cellular alterations.

Keywords: lead, toxicity, cornea, grass carp**,** *ctenopharyngodon idellus*

1. Introduction

Environment got affected by the ongoing pollution problems. The environmental effect of metals is of particular concern because heavy metals as compare to other toxicants are more toxic and effective. Due to their toxicity in relation to the aquatic organisms these are in concern because unlike organic compounds they are non-biodegradable and can only be redistributed in abiotic and biotic components. Lead is one of the known toxic heavy metals and occurs in environment in a wide range of physical and chemical forms that influence the behavior of fish adversely at concentration higher than normal. Most of the lead in the environment is in the inorganic form and exists in several oxidized states [1]. Water get polluted by both natural and human activities. Natural activities like volcanic eruptions, earthquakes, tsunamis and human activities like industrial waste, sewage waste, mining activities, chemical fertilizers, radioactive waste, urban runoff , animal waste etc.

Of the many toxicants found in aquatic system heavy metals are considered as most hazardous and become worldwide problem in recent years. Heavy metals such as lead have gained wide interest in the scientific community in recent years due to their potential health hazards, their impact on the environment is an increasing problem worldwide [2]. Among naturally occurring heavy metals, lead is a found in low concentration in the earth's crust and also found in batteries and other electrical, pigments and paints, alloys and solders, biocides, glass, catalysts, fertilizers, dentals and cosmetics, plastics, textile, refineries, fuel, used for automobiles etc when get discharged into aquatic ecosystem, which have toxic effects and can cause mortality to aquatic animals [3]. Among aquatic species, fish is a major aquatic organism. Any alternation in water quality directly affects fish, therefore fish can be used as pollution indicator directly and indirectly. As inhabitants fishes cannot escape from the detrimental effects of these pollutants [4], due to their pollution fish species have disappeared [5].

Among the inhabitants of aquatic environment, fish can be considered as most significant bioindicators of heavy metal polluted freshwaters widely documented as indicator due to its sensitivity to pollution [6]. Lead has very toxic effects on aquatic organisms upon acute and chronic exposure. The increased use of heavy metals leads to ultimate pollution which had drastic impacts on the organisms and the functioning of sense organs like eye.

Inspite of other organs of fish, eye is a key sensory system in fish due to its role in collecting and focusing images and transforming them into neural signals. Moreover, the eye has a wide surface area in continuous contact with the external medium and thus could be a relevant uptake route of metals and metalloids. Even sublethal concentrations of heavy metals are harmful to the eye of the fish and leads to cataract in fish exposed [7]. Lack of experimental works and exclusion of special senses in toxicological studies have led to deficient understanding of the role of these systems in fish toxicological injury and response. In present investigation an attempt has been made to study the effect of lead nitrate on cornea of freshwater fish, *Ctenopharyngodon idellus*.

2. Materials and Methods

Ctenopharyngodon idellus (Cuvier and Valenciennes) (length: 8$\pm$2 cm and weight: 8$\pm$2 g), was procured from Sultan Fish Seed Farm, Kurukshetra, Haryan and were acclimatized to the laboratory conditions for 15 days in glass aquarium. Water in the aquarium was renewed daily and fishes were fed with artificial feed once in a day. For acute toxicity tests, the physico-chemical characteristics of water were determined according to the standard methods [8] and were found as: temperature 22±2 °C, dissolved oxygen 8.0±2.0 mg/ml and total hardness 175±5 mg/ml, pH 7.2±0.2. 96 h LC50 of lead nitrate was determined using Probit analysis [9] and was found to be 33.27 mg/l. For chronic toxicity tests, 10 fishes were introduced into experimental tank with one-fifth (11.09 mg/l) of the LC_{50} of lead nitrate as sub-lethal concentration. Experiment was conducted for a period of 15, 30 and 45 days. At the end of each exposure period, the fish were sacrificed by cervical dislocation and eye was dissected out to remove cornea.

For histological studies tissue was fixed in bouin's fixative and was processed using standard methods [10, 11]. The processed slides were studied with light microscope at Department of Zoology, Panjab University, Chandigarh. For SEM studies corneal tissue was fixed in 4% gluteraldehyde prepared in phosphate buffer (0.2 M, pH 7.2-7.4) for 4 h, then fixed in 1% osmium tetraoxide for 1 h. After fixation it was given washings in the phosphate buffer, then downgrade in series of acetone. The dehydrated tissues were then dried in critically point dried (CPD) for 2 h, mounted on aluminum stubs and coated with colloidal gold in an ion sputter unit. Samples were viewed under Leo 435 VP Scanning Electron Microscope, IIT Roorkee.

3. Results and Discussion

Vision is an important sensory system for most fish species. Fish eyes are similar to terrestrial vertebrates like birds and mammals, but have a more spherical lens [12]. Fish normally adjusts focus by moving the lens closer to or farther from the retina. Fish eyes are broadly similar to those of other vertebrates. Light enters the eye at the cornea, passing through the pupil to reach the lens. Once light passes through the lens it is transmitted through a transparent liquid medium until it reaches the retina, containing the photoreceptors and reaches the optic nerve outside the eyeball.

Cornea is the external most layer of the eye, which allows the light to enter the eye and bends them so that they can be brought to a focus on the retina. Fish cornea consists of five intact layers. Anterior most layer of cornea consists of squamous epithelium cells covering the lower basement membrane followed by substantia propria or stromal layer. Corneal endothelium formed the posterior most layers resting on descemet's membrane.

Cornea of *C. idellus* studied under light microscope showed normal histology of cornea i.e. outermost squamous epithelial 4-5 layers thick and continuous, epithelial cells having round nuclei, acellular stromal layer composed of collagen fibril mat that run parallel to each other and it formed the major part of the cornea, also having keratocytes scattered in the whole fibril mat and innermost endothelial layer was also 4-5 layers thick and the endothelial cells have round nuclei (Fig I A-C).

But after 15 days exposure to the toxicant, slight sloughing off of the epithelial layer, necrotic epithelial cells, change in the shape of the nuclei, degenerated stromal layer and fibrillar mat were observed (Fig II D-F). Sloughing was also demonstrated by other workers [13], in larvae of striped bass *Morone saxatilis* that were exposed to copper. Number of keratocytes decreased, endothelial layer showed slight erosion, endothelial cells have undergone necrosis. Similar changes were also reported [14], in brook trout, *Salvelinus fontinalis* (Mitchill) exposed to acute and chronic levels of pH.

As the exposure time increased to 30 days, the cornea depicted increased damages as increased sloughing off of the epithelial layer was seen (Fig II G-I). Other workers [15, 16] investigated affect of linear alkylbenzene sulfonate and TBT (Tributyltin) toxicity, also reported sloughing of epithelial layer due to to the visual functions of Tigerperch (*Terapon jarbua* Forsskal) respectively. Epithelial cells showed severe necrosis, increased degeneration of stromal fibres, keratocytes number decreased and existing ones showed change in shape. After the exposure period of 45 days, sloughing off of the epithelial layer, necrosis of the epithelial cells, extreme change in shape of the nuclei, degeneration of the fibrillar mat become severe and erosion resulted in complete destruction of the endothelial layer (Fig II J-L). Similar observations like leak in corneal epithelial cell membrane due to the toxicity of benzalkonium chloride and gentamicin has been reported [17].

For Scanning Electron Microscopic studies, among the five layers of cornea anterior layer i.e., corneal epithelium has been studied to determine the impact of the toxicant, it is the outermost layer of the eye and comes in direct contact with the contaminants. It is a thin multilayered tissue, having fast-growing cells, due to the continuous growth, shedding occurs constantly, epidermal region having well-defined microridges surrounding small structures the microplicae, are characteristic of the Osteichthyes.

SEM studies of controlled fish cornea showed normal features like, anterior region of the cornea is flat and peripheral region is thicker whereas the central region is thinner, epithelial layer showed regeneration at the central region and due to the continuous regeneration there was shedding of mature cells, Anterior region composed of pavement cells, has microridges which trap the mucous in the intramicroridgal space, at higher magnification, the central part of the epithelial region showed prominent microridges filled with mucous, due to regeneration the older epithelial cells or the mature epithelial cells sloughed off, beneath the anterior epithelial membrane porosity and vacuolization was found and the regenerated epithelial cells appeared elongated (Fig III A-C)

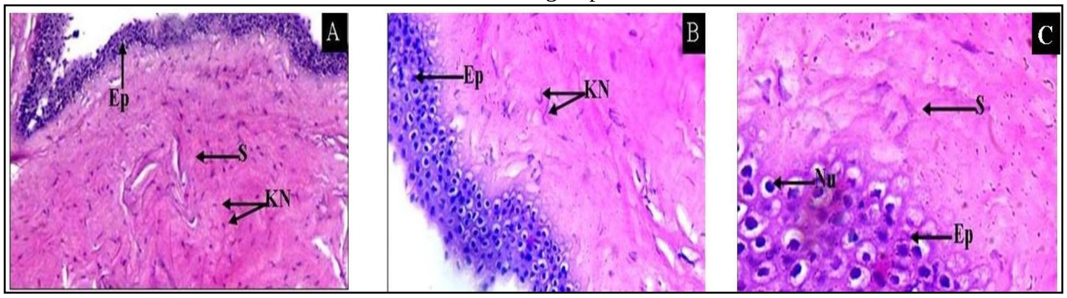
After 15 days exposure of the toxicant to the fish, the cornea showed some changes in peripheral region which became thicker and hardened than the normal structure; central region showed shrinkage and cracks and wrinkles appeared. The peripheral region which is thicker than the central region appeared more affected (Fig IV D-G). In corneal magnification, the epithelial layer close to the centre had microridges, having mucous entrapped microprojections of two elasmobranch species, the dogfish and skate [18]. Central region of epithelial layer got uplifted and the epithelial cells appeared shrinked, due to uplifting of upper layer the lower layer showed formation of globule like structures, a large no. of irregular shaped cells appeared in the epithelial layer which partially detached from the surface and form a lump of detached cells.

Increasing the exposure period to 30 days cornea showed increased damage as at higher magnification, the apical cells of the epithelial layer shows similar pattern and a number of cells appear degenerated, microridges got ruptured intensively and got filled with mucous (Fig IV H-K), due to thinning and shrinkage the distance between the microridges also increased, anteriormost region of epithelial layer showed sloughing off also noticed by the other worker [16] in Tigerperch. Sloughed epithelial cells form globular structures.

After 45 days exposure of the fish to the toxicant, changes in cornea showed, severely degenerated cells of the apical region at higher magnification, amount of mucous entrapped in the microridges of epithelial region increased severely, distance between the microridges increased more due to the severe thinning and shrinkage of the microridges, irregular shaped cells of epithelial layer increased and formed lumps at various regions, epithelial cells formed globular structures (Fig IV L-O). Similar observations made by other workers [19] in trout and *Cyprinus carpio* respectively.

4. Figures

Control group



Abbreviations:
Ep- Epithelium, S- Stroma, KN- Keratocyte nuclei.
On exposure to lead nitrate

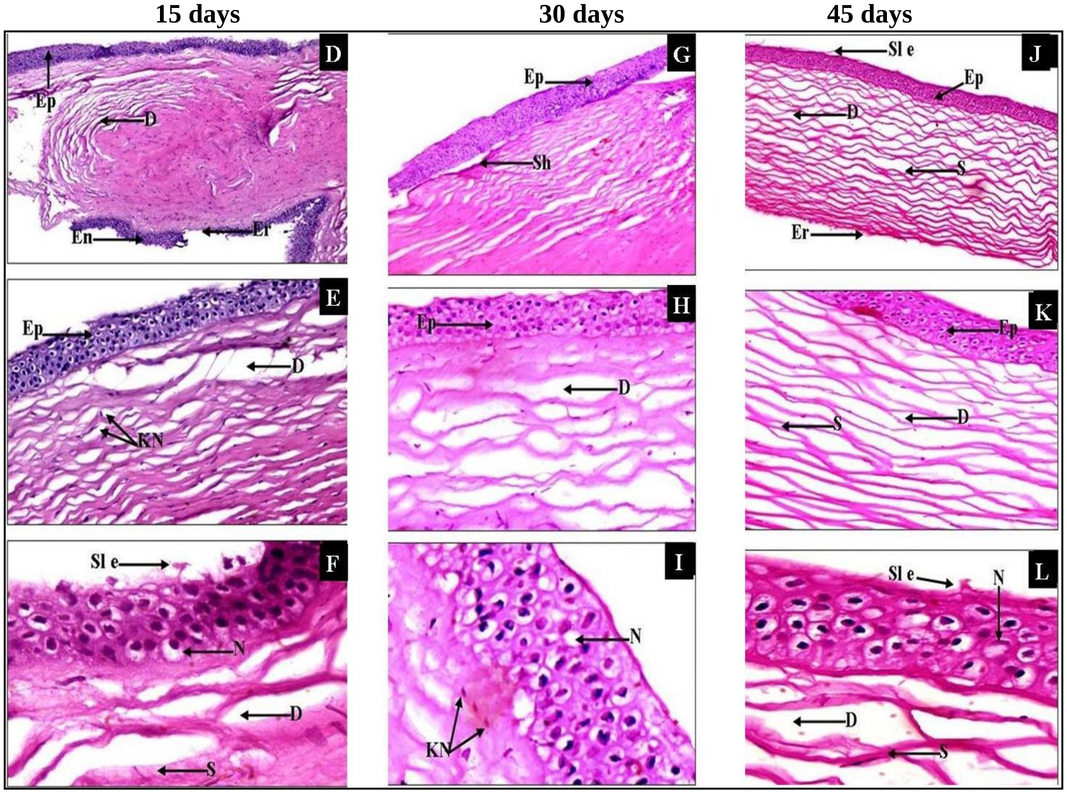
Fig I: Photomicrograph of cornea of *Ctenopharyngodon idellus* of control group (A-C: 10X, 40X, 100X)

15 days **30 days** **45 days**

10X

40X

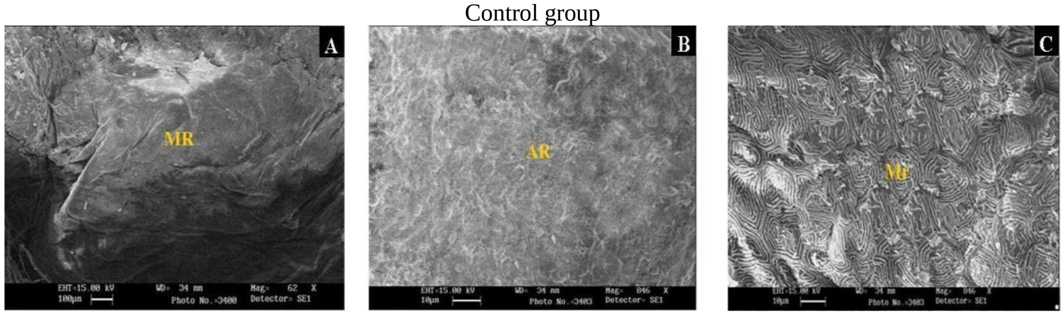
100X



Abbreviations:
Ep- Epithelium, S- Stroma, En- Endothelium, KN- Keratocyte nuclei, D- Degeneration of stroma, N-Necrosis of epithelial cells, er-Erosion of epithelial membrane, Sl e- Sloughing of epithelial layer.

Fig 2: Photomicrograph of cornea of *Ctenopharyngodon idellus* on exposure to lead for 15 days (D-F), 30 days (G-I) and 45 days (J-L).

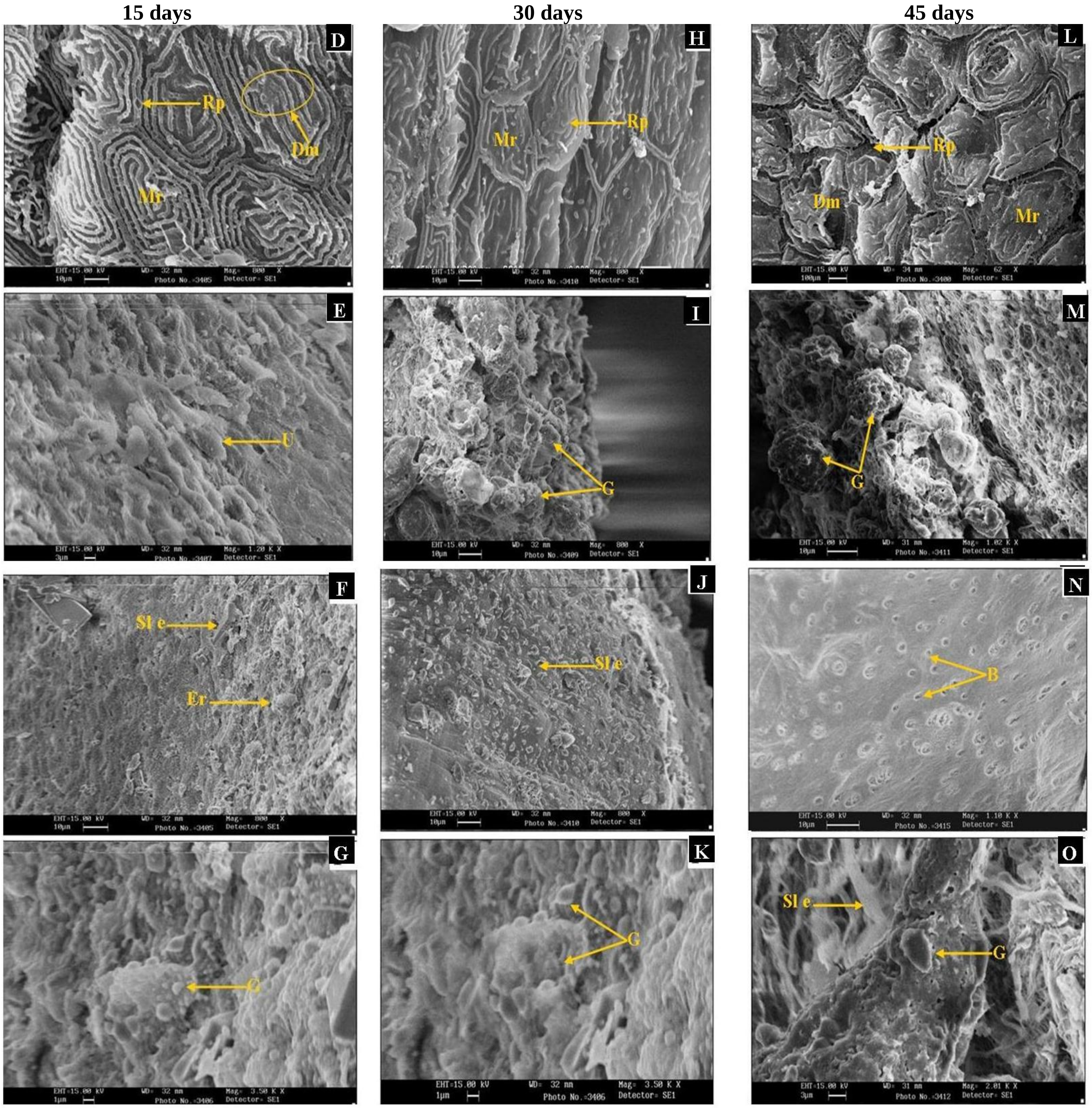
Control group



Abbreviations: MR- Middle region, AR- Anterior region, Mr-Microridges.

Fig 3: Scanning electron micrograph of cornea of *Ctenopharyngodon idellus* of control group

On exposure to lead



Abbreviations:
MR- Middle region, AR- Anterior region, Rp- Rupturing of microridges, Er-Erosion of epithelial membrane, Sl e- Sloughing of epithelial layer, Mr-Microridges, Dm- damaged microridges, U-Upliftment of epithelial cells, G-Globular structures.

Fig 4: Scanning electron micrograph of cornea of *Ctenopharyngodon idellus* on exposure to lead for 15 days (D-G), 30 days (H-K) and 45 days (L-O).

5. Conclusion

The present study of cataracts in lens of eye of *Ctenopharyngodon idellus* would be useful information about the impact of contaminants on eye tissues like lens and alterations in the structure of the lens leads to severe vision impairment. From the present findings, it can be concluded that prolonged exposure of lead to the fish has severe effect on cornea, lens and optic nerve. Thus, it is determined that lead concentration above the threshold level could be deleterious to aquatic life particularly to fishes and may harmfully affect human life.

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7. References

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