Effects of Chronic Exposure to Lead on Oocyte Maturation in Heteropneustes Fossilis

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Abstract—*There is an increasing concern that environmental chemicals such as lead have a direct effect on fertility. Sub lethal concentration of lead acetate i.e. 3.0 mg/litre for six weeks produced significant changes in the oocyte of fish.*

Keywords*: Lead, oocyte, sublethal, chemicals*

1. INTRODUCTION

Fishes are important alternative to solve the food problem as they provide assmilable food for mankind. It is therefore, significaant to find out the causes of reduced fish fertility rate that mainly resulted into less fish production.

Lead's hazards to the reproductive process have been known for at least a century. During the industrialization of Europe, reproductive failures and congenital lead poisoning were described by Paul (1860). Workers recognized sterility, abortion, stillbirth and premature delivery as common, not only among female lead workers, but also among the wives of men who worked in the lead trades (oliver, 1911; Hamilton and Hardy, 1949; Lane 1949). Lead exposure is associated with a number of reproductive defects. The lead dispersed through gasoline exhausts, and peeling paint etc. never fully disappears from our environment nor has man evolved a good biological system to offer any protection from it. Ezra Susser and his colleagues at Columbia University in New York found that children who had been exposed to high levels of lead in the womb were more than twice as likely to go on to develop Schizophrenia.

Fish readily absorbs dissolved heavy metals in the water. Any damage caused by chronic exposure to lead in hormone producing cells of ovary may adversely affect the fish production.

Adeyemo (2008) observed the histological alternations in the ovaries of clarias gariepinus exposed to environmental lead. Upadhyaya and Haidar (1986) observed the germinal vesicle break down in oocytes of cat fish, Mystus vittatus. Mayer et al (1988) studied the aspects of reproductive biology of the bass (Dicentrarchus labrax).

The aim of present investigation is to study the effects of lead acetate on the oocyte maturation in Heteropneustes fossilis.

2. MATERIALS AND METHODS

Living specimens (approx. 14 to 16 cm in length and approx. wt. 70-80 gm) of fresh water teleost fishes, *Heteropneustes fossilis* (Bloch) were collected from the unpolluted fresh water, resources of the Hastinapur (U.P.) and were acclimatize to the laboratory condition for 4 to 5 days. Prior to experimentation, fishes were treated in 1% potassium permanganate solution for 15 minutes to disinfect the fishes. Fishes were maintained in laboratory glass aquaria in dechlorinated tap water (pH=7.4, hardness 160 ppm (as $CaCo_3$), alkalinity 87ppm). Fishes were fed twice daily with commercial fish pellets and small aquatic animals. Water temperature maintained between 18^0C to 24^0c for six weeks.

The fishes were exposed to sublethal concentration *i.e.* 3mg/L lead acetate according to the 'standard Methods (1971) of the American Public Health Association. In each experimental group, 20 fishes were treated with the calculated dose of the lead acetate and a second group of 20 fishes in dechlorinated tap water served as control. After completion of tenure both the groups of fishes were processed simultaneously.

3. TOXICANT USED

Lead acetate of analytical grade was selected as the toxicant, obtained from BDH, England.

4. METHODS OF HISTOPATHOLOGY

4.1 Light microscopic studies

Ovaries from both the groups were taken out, washed in 1% saline solution to eliminate mucus and blood deposits and after cutting in pieces of required thickness fixed immediately in 10% buffered neutral formalin and alcoholic Bouin's fluid for 12 hrs. Standard methods of dehydration, clearing and embedding were used. Serial sections of 5-6 µ thickness were cut and stained with Delafield haematoxylin and alcoholic eosin.

4.2 Electron microscopic studies

The ultrastructural studies were carried out in Regional Electron Microscope Facility at all India Institute of Medical Sciences, New Delhi.

For ultra structural studies fishes from both the groups were dissected ventrally and heart was exposed to perfuse intracardially with normal saline followed by 3% gluteraldehyde (GA) solution in 0.1 M phosphate buffer for about 10 minutes to give proper preservation. Ovaries were washed in 0.1 M phosphate buffer, and placed it over night at 4^0C. Final trimming of the ovaries to appropriate size was done in the buffer. The trimmed tissues of about 1mm thickness were post fixed in 1% O_sO_4.

Further processing of tissues was done in the All India institute of Medical Science, New Delhi.

5. OBSERVATION

5.1 Control

Histologically, ovaries of this teleost fish are of open type, each having an ovocoel and covered by three layered wall. Various stages of developing oocytes are arranged on either side of the ovigerous lamellae. Immature oocytes tend to occur towards the periphery of the ovigerous lamellae, characterized by their small size, presence of large nucleus and absence of yolk and older ones are centrally placed. The ooplasm remains unstained in early stages but later when the quantity increases; it shows a strong affinity for basic dyes. Also at later stages, the number of nucleoli increases to six to eight and these are arranged along the periphery of the nuclear membrane.

The thickness of the oocyte wall varies from immature stage to mature stage.

The maturing ova are characterized by the formation of yolk. The number and size of yolk vesicles increases in the ooplasm. Further development of the oocyte is marked by the formation of a thin layer of follicular cells around the ooplasm. Nuclear membrane shows a wary margin and forms pocket like structures in which are found the nucleoli. Matured ova are characterized by the disappearance of nuclear membrane and excessive deposition of yolk. Oolemma becomes prominent and theca externa and theca interna are clearly recognized. There is greater succession in the size of mature ova.

6. LEAD ACETATE TREATMENT

Maturing oocytes were reduced in size and interfollicular spaces were increased. The different layers of mature oocytes showed degeneration. Reduction in the quantity of yolk of the maturing oocytes. Germinal epithelium became thinner and was reptured at various places. Electron microscopic observations in the ovary were vacuolation of cytoplasm, reduction in the number of golgi apparatus and shrinkage of nuclear material in the secretory cells of interstitial tissue. Apoptosis/necrosis of thecal and granulosa cells of follicular layer was observed.

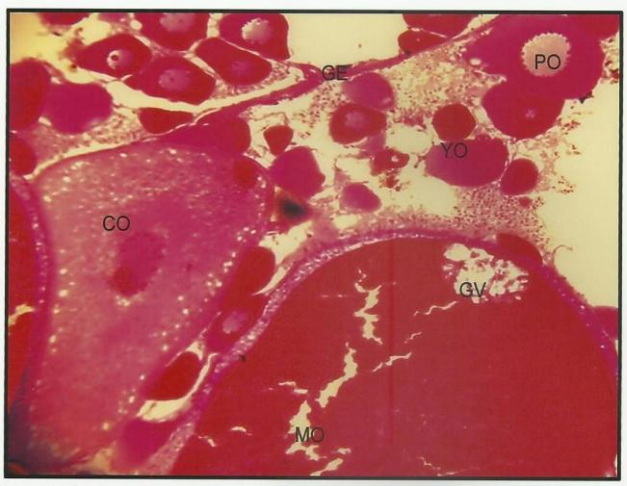

Fig.19.Section of ovary of fish from control group showing normal structure of different stages i.e. mature, cortical stage, perinuclear and young oocytes. H/E Stain , X-100

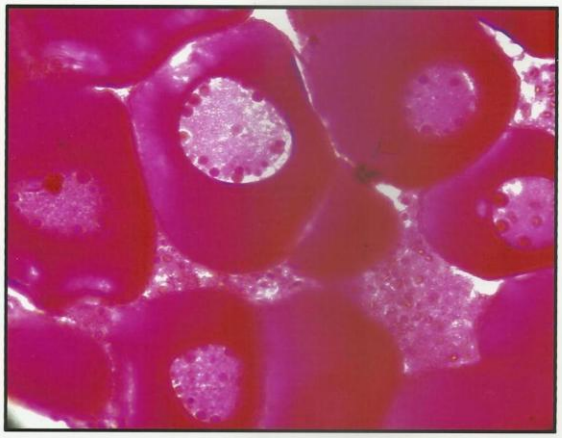

Fig.20. Perinuclear oocytes showing normal structure having many nocleoli from ovary of control fish. X-240

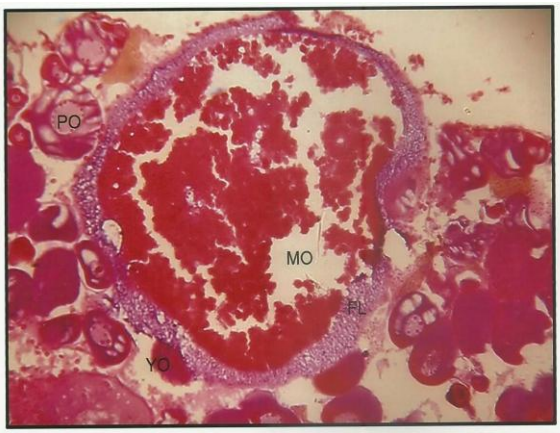

Fig.21. Transverse section of ovary of Heteropneustes fossilis exposed to 3mg/l of lead acetate for six weeks showing severe degenerative changes in follicular layer including ooplasm of mature follicle, and primary/young oocytes. H/E Stain, X-100

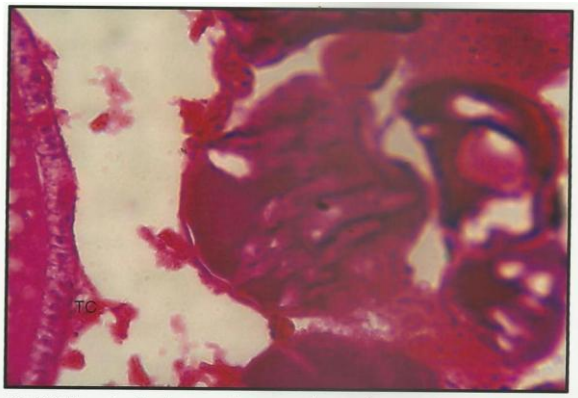

Fig.22. Thecal cells degeneration, granulosa cells necrosis, oocytic degeneration and blood filled spaces are seen in the ovary of lead treated fish. H/E Stain, X-400

7. DISCUSSION

Calcium is important for growth, reproduction and many other physiological processes. In full grown animals in flux and efflux of calcium ions are in equilibrium, whereas during periods of growth and reproduction net calcium accumulation occur. During such periods large amount of calcium ions are transported from the sites of entry through the blood and other compartments of the ECF to bones or ovaria.

GTH acts on ovarian follicle cells of post vitellogenic phase and causes MIH synthesis and release (Nagahama, 1987b). Production of this steroid is effected via the interaction of two follicular layers, the thecal and granulosa layer (Young et al. 1986).

Lead can cross the cell membrane in various ways. Once it has penetrated the cytoplasm, lead continues its destructive mimicking action by occupying the calcium binding sites on numerous calcium-dependent proteins. Steroidogenesis in fish, although induced by GTH, directed in two predominant forms; 17 β-estradiol and 17α, 20β-dihydroxy prognene-3 one (17α, 20β-DP). 17β-estradiol has a peak during vitellogenic stage, while 17α, 20β-DP is the major steroid in post-vitellogenic phase or phase of final maturation. 17α, 20β-DP binds to oocyte membrane and produces signals for final maturational event.

Chronic exposure to lead severely damaged gonadotrophs of pituitary of H. Fossilis. As GTH binds to theca and granulose cell receptor in the ovarian follicle and thereby increases steroidogenesis, any damage in gonadotrophs may results into inhibition of secretion of GTH and so that steroidogenesis. Reduction in synthesis of 17β-estradiol may hamper oocyte growth due to inhibition of vitellogenin formation and deposition in the oocytes. Similarly, reduction in synthesis of 17α, 20β-dihydroxy pregnene-3 one (MIH) resulted into failure of oocyte maturation.

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