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METAL - INDUCED INTRINSIC RESPONSE OF THE FEMALE CICHLID ETROPLUS MACULATUS (BLOCH) FROM SOUTH WEST COAST OF INDIA

BY

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ABSTRACT

The manifestation of the toxic effect of the metals copper, mercury and selenium on the ovarian development of an estuarine fish ***Etroplus maculatus*** *at sublethal levels have been studied. Of the five maturity stages identified, three (immature virgins, maturing and ripe) were considered for toxicity studies. On subjecting the ovary to histological examination after 96h of exposure to the metals in different experiments, it was observed that mercury caused a deleterious effect on all the three stages of ovarian maturity. Selenium and Copper did not induce any structural variations to the first stages of maturity. However, drastic changes could be seen in the second and third maturity stages.*

INTRODUCTION

Copper and Selenium are two essential dietary trace elements in fish, which could function as potential water borne toxicants at elevated concentrations (Hunn ***et al.***, 1987).Copper concentrations including both dissolved (2.24-22.2μg/1) and particulate forms (44-298 ppm) in the Cochin estuary has been reported by Ouseph, 1992. Selenium, identified both as a toxicant (Niimi and LaHam, 1975) and as an essential micro nutrient (Hilton ***et al.***, 1980) for fish finds its way into the aquatic realms as a result of geochemical processes. Its occurrence in the bivalve ***Villorita cypronoides*** var ***cochinensis*** sampled from

Cochin backwaters has been reported by Geetha (1992). Mercury has been implicated as a highly toxic metal. Mercury concentrations reported from the aquatic system around Greater Cochin (0.12 to 0.95) has served to induce more rigorous and systemic investigation. A realization of the potential hazard that these metals pose to fishes, prompted the initiation of this study on the toxic effects manifested at the sublethal level on the estuarine teleost ***Etroplus maculatus***. An attempt has been made to study the histological effect of copper, mercury and selenium on the ovarian maturation of the teleost, ***E. maculatus***.

MATERIALS AND METHODS

The animals were collected from the backwaters of Cochin (Lat. 9°58' & Long. 76°81') and maintained in the laboratory in well-aerated, dechlorinated, tap water. The fishes were fed with shredded clam meat and boiled egg white. The water in the aquaria was changed daily. The fishes were acclimatized thus for over a week, before the commencement of all experiments.

Toxicants used

Copper, mercury and selenium were the three metals used. 1g of scrupulously cleaned metallic copper (dissolved in a minimum quantity of dil. nitric acid), 1.3539g of mercuric chloride and 1.406 of selenium dioxide was made up to 1000 ml each in deionised distilled (Milli Q) water to obtain 1000 ppm stock solutions. The test concentrations were prepared by appropriated diluting the stock solutions.

Toxicity Studies

All tests were conducted in glass troughs, by static renewal bioassays. Well-aerated, dechlorinated, tap water was for all experiments. The physico-chemical parameters of the test medium were __ temperature: 28°C ± 2 ; ph: 6.8 ± 0.1; dissolved oxygen: 7.2 ml/l. The water in the troughs was changed daily to replenish the oxygen content and to maintain appropriate concentrations of the toxicant. Feeding was stopped 24h prior to the commencement of the experiment.

Metal induced changes on the ovary of fishes were assessed through sublethal toxicity studies. The metal concentrations corresponding to $1/10^{th}$ of the LC_{50} (Litchfield and Wilcoxon, 1949) values were chosen as the respective

used for the three metal -copper: 0.005 ppm; mercury: 0.013 ppm and Se: 0.462 ppm.

The acclimatized fishes were exposed to sublethal concentrations of the toxicants. Duplicates and controls were also maintained. A sufficient number of fishes were exposed in each trough so that at least three fishes of the same age/maturity stage could be sacrificed after 96h of exposure. The ovaries were removed and examined histologically by using Hematoxylin and eosin.

RESULTS

Figures 1a, 2a and 3a gives the normal chromatin - nucleolus, perinucleolus and yolk stages. During the sub - lethal exposure period, the animals did not exhibit any signs of stress outwardly.

The ovaries of the fishes were observed to be severely affected by 96h of exposure to the sublethal concentrations of the toxicants. Histological examination of the chromatin - nucleolus stages, perinucleolus stages and secondary yolk stages, representing the different maturity stages (I, II and III) of the females revealed that the three metals adversely altered the morphology of the ovary and each metal acted differently.

Copper did not cause any deleterious effect on the structure of the oocyte in the chromatin nucleolus stage (Fig.2a). However the oocytes of the perincleolus stage (Fig.2b) were observed to be highly vacuolated. Fragmentation of the cell membrane was a prominent feature. In the secondary yolk stage (Fig.2c), copper was observed to have caused total rupture of the cell membrane with the release of yolk granules.

On exposure to mercury, oocytes in the chromatin - nucleolus stage (Fig.3a) were observed to have crumpled in contrast to copper toxicity. Mercury did not have any effect on the cell membrane of oocyte and disintegration of yolk granules were observed. Complete hypertrophy of yolk granules were noticed in the yolk stages (Fig.3a) with only fragments being left behind.

Selenium, like copper, did not seem to have any deleterious effect on the chromatic in-nucleolus stage (Fig.4a) as no change was observed from that of

the normal ones. Hypertrophy of the yolk granules of the perinucleolus stage (Fig.4b) was observed on exposure to selenium. Cell membrane was seen to have ruptured. Vacuolation of oocytes and rupture of cell membrane was prominent in the yolk stage(Fig.4c).

DISCUSSION

From the light microscopic observation of the ovary, it was seen that copper and selenium did not cause any change to the chromatin-nucleolus stage. All the same, alterations especially the rupture of the cell membrane, noticed in the perinucleolus stage and yolk stages are noteworthy.

Copper is suggested to have acted on the oocytes by dissipation of an oxygen permeable barrier located in the egg membrane (Akberali and Earnshaw, 1984). Selenium exposure to the teleosts - ***Lepomis cyanellus*** (green sunfish) and ***Lepomis microlophus*** (redear sunfish) resulted in leakage from the zona radiata which appeared markedly indistinct (Elsie ***et al***, 1982). A marked increase in the number of atretic follicles (i.e. maturing follicles which degenerate insitu) was also observed. The necrotic process was advanced in some follicles. Vacuole coalescence occurred and the number of fat vacuoles appeared to be increased as compared to the normal ovaries. Similar observations of vacuolation and increase in lipid droplets were also evident in the case of ***E. maculatus.***

Low accumulation of selenite in the gonadal tissue of juvenile fish was observed in comparison with adults (Lemly, 1982). this supported the observation of the present study in which the earlier stages remained unaffected even after exposure to selenium. Further, Cumbie and Van Horn (1978) observed a three fold accumulation of selenium in the ovaries of ripe females of sunfish as compared to their muscle tissue.

The 96h exposure to mercury was observed to have caused the most deleterious effect on the different stages of ovary. A marked feature was its ability to enter the oocytes (probably with the help of cell membrane mediators) and to cause chaos once within.

Mercury in known to bind strongly with Sulfhydryl groups, and it has been to be more toxic during embryo (Nriagu, 1979). The major processes affected might include cellular differentiation, proliferation and inhibition of mitotic cell division (Friberg and Vortal, 1972) to produce chromosomal aberrations, polyploidy and somatic mutation with gonadal accumulation etc., all of which substantially impair development.

Reduced viability of Steelhead trout sperm following direct treatment with mercury foe 30m as observed by McIntyre (1973), damage to both eggs and sperms by chronic exposure of the brook trout, ***Salvelinus fontinalis***, to mercury as observed by McKim ***et al.***, (1976) and in ***S. namaycush*** as observed by McCrimmon ***et al.***, (1983) lends further support to the present findings.

Hypertrophy of oocytes at sublethal levels indicates that, even at lower concentrations, the chances of producing high embryonic and larval mortality and complete inhibition of reproduction are relatively high. Mercury was observed to be teratogenic to virtually all classes of vertebrates (Weis and Weis, 1977). Formation of anomalous offsprings (partial to nearly complete twinning) of rainbow trout, bass, catfish etc., were reported to occur more frequently with mercury than tests with copper or most other metals. As mercury exposure resulted in completed hypertrophy of the ovary of ***E. maculatus*** at sublethal concentrations (0,013 ppm), teratogenic effect of inorganic mercury on development in ***E. maculatus*** like reduced axis formation, fore brain defects, cyclopia and anophthalmia (Nriagu, 1979) could probably occur only below this concentration.

In conclusion, it could be inferred that although, based on the LC_{50} values, copper was seen to be the most toxic metal (the order of toxicity to E. maculatus, being, Cu > Hg > Se), histological examination of the ovary after sublethal exposure revealed mercury to be the most toxic of the three metals.

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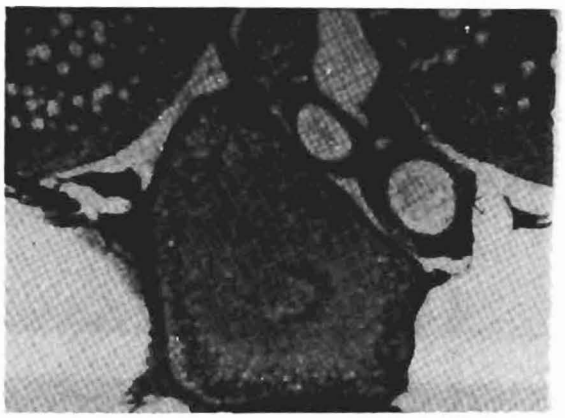
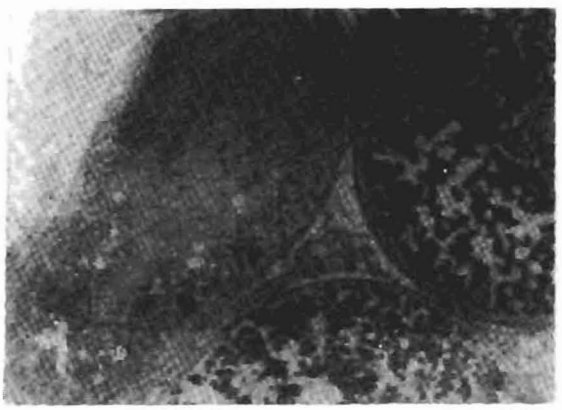
Abbreviations

1d- lipid droplets
yn-yolk nucleolus
yg-yolk granules
rcm-ruptured cell membrane
vc-vacuolisation

Fig 1.





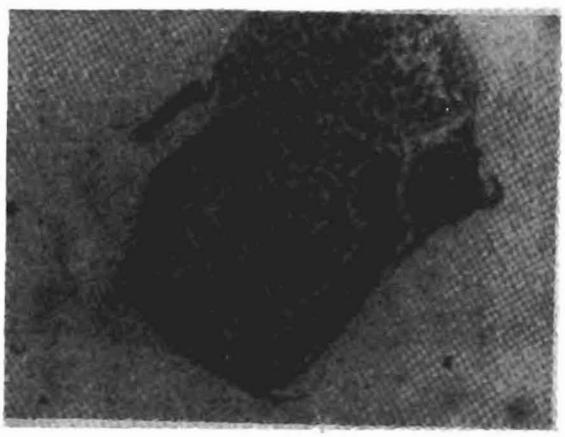


Fig 2.

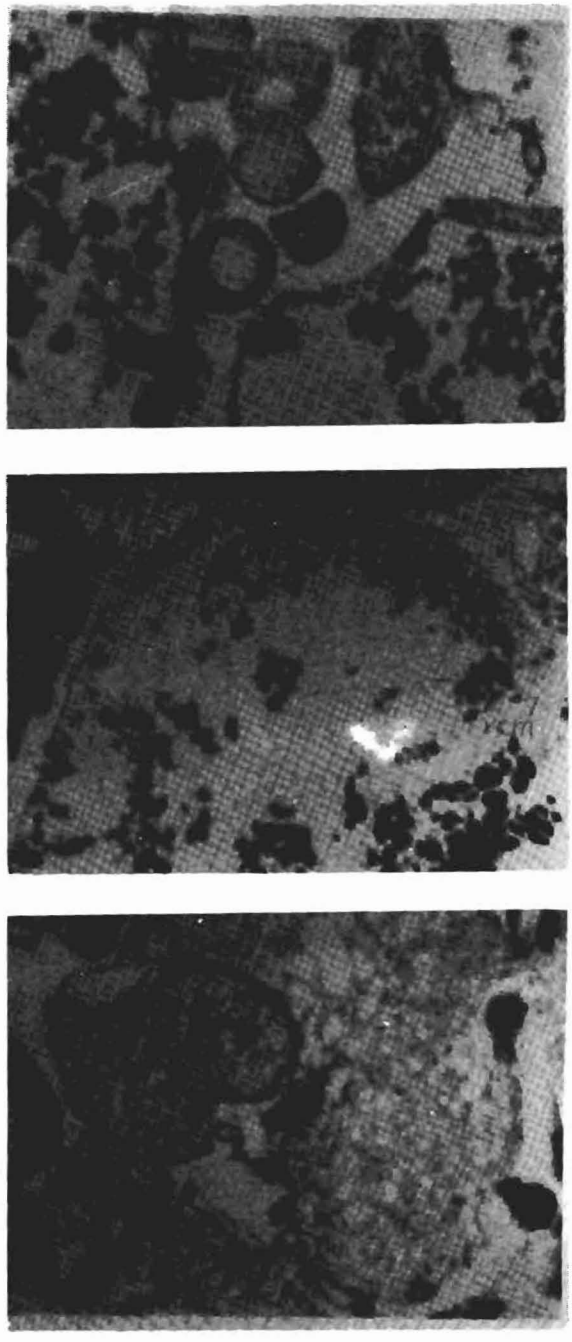


Fig 3.