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Research Paper

Selenium protection against cadmium toxicity in gonadal axis of freshwater Indian catfish *Clarias batrachus*

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Abstract: Selenium is an essential micronutrient for human beings and fresh water animal. The Selenium is known to control the aquatic environment and protect injury to be animals. Protection of the aquatic environment by Selenium is an important function and has attracted the attention of scientists presently all over the world.
The advancement of civilization has resulted in an increasing technological use of heavy metal in industries pollution by Cadmium is one of the most important health hazard all over the world. Cadmium present in the water bodies enter into the body of fishes by gills and general body surface and producing toxic effects.
The present study was designed to investigate the acute effect of Cadmium in the testis of fish Clarias batrachus. In order to elucidate the effect of supplementation of selenium, on protective role against Cadmium toxicity was observed. The abnormalities were seen in the testis of Cadmium exposed fishes the seminiferous lobules exhibited marked degeneration of germinal, elements spermatids and spermatogonia. The cytoplasm displays vacuolization. Selenium supplemented group showed disappearance of vacuolization and reappearance of chromatin material. The result presented here indicate that Cadmium chloride is susceptible to testis of Clarias. Selenium produced significant positive influence of reproductive system. This reappearing mechanism strongly suggests in the protective mechanism of Selenium treatment against the acute Cadmium toxicity.
Keywords: Cadmium, Selenium, Fish Protection.

INTRODUCTION

Metals are an intrinsic component on earth's crust. With the rapid development the evolution of metal based industries has led to the contamination of environment with heavy metal. This has led to the increased requirement and exposure of man and ecosystem to the toxic levels thus presenting two potential health hazards to man as well as aquatic life. Strict controls have been laid down and top priority is being given in setting up the safe limits to which the population may be exposed. The use of

cadmium from sludge contributes to environmental pollution. Most of it gets deposited in the soil or water.

Thus, it is evident that mankind is exposed to cadmium via food, water and air. There is abundant evidence to indicate that cadmium accumulates in significant concentration in different organs with age. The liver and kidney are two main organs where cadmium accumulates; besides this, pancreas and spleen also large amount of cadmium (Nordberg and Nishiyama, 1972).

The survey of literature on cadmium toxicity clearly shows that this heavy metal is implicated as a causeative agent of several pathological disorders both in laboratory animals and human being (Anil Pillai et al., 2002 and Aruna et al., 2006).

Heavy metals represent some of the most important pollutants posing different problems for the environmental protection because they cause hazardous effects on aquatic life and are least degradable substances. Heavy metals and their salts form an important group of environmental, pollutants all over the world. These metals and their salts may originate from geological weathering, industrial processing of ores and metals, from garbage and solids waste dumps and by animal and human excretions which contains certain amounts of heavy metals.

Heavy metals may be categorized mainly into two groups on the basis of their role played in animals. The first group consists of metals essentials for life activities like zinc, copper, iron, cobalt, manganese and second group includes those metals which do not play any essential role such as mercury, cadmium, arsenic and lead etc. In contrast to most organic substances the non essential heavy metals are not usually eliminated from the aquatic organisms.

The release of increasing quantities of heavy metals into the water resources endangers the life of aquatic fauna.

Cadmium, a crystalline, silver-white malleable metal is obtained as a byproduct in refining of zinc and other metal.

The atmosphere plays an important role in the dispersal of cadmium within the environment.

Toxicity of different pollutants has already been studied by numerous scientists. The knowledge regarding the details of toxic effect of heavy metals on reproductive physiology in fish is still in adequate and fragmentary. The studies on fish reproduction in relation to heavy metal toxicity, will help Pisiculturist and fish biologist to increase fish production as the reproductive functions are controlled directly or in directly by hormones from hypothalamus and pituitary gland.

Protection of aquatic environment by selenium is an important role and has attracted the attention of scientists presently all over the world. The beneficial effects of selenium against cadmium toxicity have several interesting features. Selenium is a trace element essential for the activity of glutathion peroxidase which catalyses the detoxification of hydrogen peroxide and organic hydroperoxides (Hogberg and Alexander, 1986)

Selenium is an essential micronutrient for" humans and fresh water animals, the selenium is known to control the aquatic environment and protect injury to the animals (Hogberg and Alexander, 1986; Diplock, 1993 and Richard *et* a*l*., 1998, Singh 2008).

It is generally derived through food chain from soil where it is unevenly distributed is low and high levels. Though a voluminous works have been done on the fish toxicity and histopathological impact to different organs, less attention has been paid to the impact of selenium on the gonads and pituitary.

Toxic chemicals which reach the various water resources during the different control

operations, make a considerable impact on fresh ecology in the are under treatment.

Ohta and Imamiya (1986) have studied the acute toxicity in testicular damages and observed that the resulted necrosis and gonadal atrophy are remarkably migrated by simultaneous selenium treatment.

Teleost fishes are commercially important culturable air breathing catfish which shows clear temporal organization in their reproductive activity. A majority of teleosts are seasonal breeders, while others breed continuously.

In the present study an attempt have been made to investigate the acute effect of cadmium, at safe concentration in testis of fresh water fish Clarias batrachus. In order to elucidate the effect of supplementation of selenium, on protective role against cadmium toxicity were also observed.

MATERIALS AND METHODS

Fresh water Indian catfish *Clarias batrachus* weighing around 55-60g and average length of 18-20cm were procured from local Lake of Sagar, M.P. Fish were transferred into experimental glass aquaria (size 18"12"9"). Aquaria water was changed daily during the acclimatization period. The fishes were fed adlibitum with dried shrimp power daily.

The present study was designed to investigate the acute effect of cadmium at safe concentration in gonadal axis of *Clarias batrachus.* In order to elucidate the effect of supplementation of selenium, on productive role against cadmium toxicity were also observed. After acclimatization fishes were transferred into five separate exposure aquaria. Each aquaria was filled with 10 litre of dechlorinated water.

The first, group kept as control. Second and third group of fishes were exposed with cadmium chloride at the concentration of 10 ppm for 20 and 40 days.

Fourth and fifth groups of fish were exposed with cadmium chloride at the same concentration as described earlier groups and supplemented with selenium (sodium selenite, Na_2SeO_3) into exposure media at 0.1 ppm concentration for the same time period as described above for earlier groups.

Cadmium chloride a heavy metal compound as obtained from Loba Chemical, Bombay the exposure concentration of cadmium chloride was established on the base of LC_{50} value for 96 hours. The LC_{50} value obtained was 28 ppm. The LC_{50} value was conducted by according to the standard method of (APHA, 1981). Sodium selenite was purchased from CDH (P) Ltd. Bombay.

The testes of the fish from different experimental group were dissected out at 20 and 40 days during the experiment. The total length of fish, body weight and the gonads weight were also recorded.

The tissues were kept for 24 hours in fixatives. After removing the fixative solution tissues were then dehydrated in different grade of alcohol cleared in methyl benzoate and benzene and was embedded in paraffin wax. The sections approximately 6µ were cut by using a rotatory microtome and sections were stained with suitable stains (Mallory's striple stain).

RESULTS

Histology of gonads: The gonads play an important role in the reproductive physiology of all animals. In the *Clarias batrachus* studied, exposure to cadmium chloride results in decreased testicular and ovarian weights. Selenium supplemented group showed significantly ($p<0.05$) increased testicular and ovarian weights when compared with cadmium chloride exposed fish.

Testes: Normal testes shows enclosed in a peritoneal membrane below which lies a

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thick layer of connective tissues fibres and seminiferous epithelial cells.

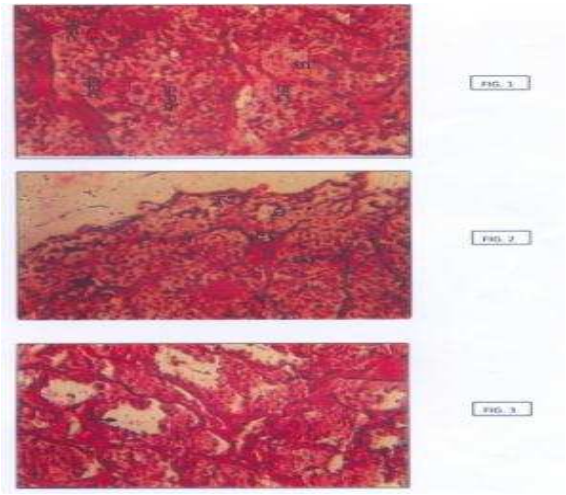

Photo micrographs of Median Transverse sections of Testes of *Clarias batrachus*
Abbreviations: Epithelial cells (EC), Interstial tissue (IT)**,** Primary spermatocytes (PSC)**,** Spermatids (S)**,** Spermatozoa (Sp), Seminiferus lobules (SL), Spermatogonia (SPG)**,** Secondary spermatocytes (SSC)

Fig.1: Section showing histology of the normal testes. (450x).
Fig. 2: Section showing histology of the controlled testes. (450x).
Fig. 3: Arrows showing degenerative changes after 20 days exposure of cadmium (450x).

In the testes a number of seminiferous lobules of various sizes are present (fig.1) the seminiferous lobules are closely packed with different stages of spermatogenesis.
Testes of 20 days control fish (fig.2) showed no marked changes were observed in the testes, when compared with the testes of control.
Cadmium exposed (10 ppm) (fig.3) fish produced the degenerative changes in the testes.

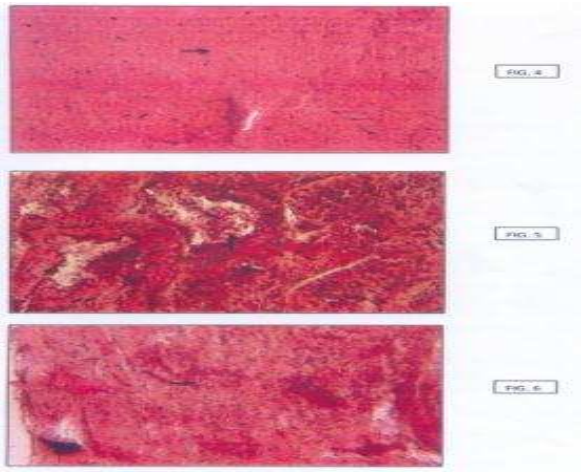

Fig. 4: Arrows showing slightly improved germinal epithelium after 20 days exposure of cadmium and supplementation of selenium. (450x).
Fig. 5: Arrows showing degeneration of germinal elements after 40 days Cadmium exposure.
Fig. 6: Arrows showing reappearance of chromatin material after 40 days exposure of cadmium and supplementation of selenium. (450x).

Selenium supplemented group (fig.4) showed slightly improved germinal epithelium was also observed.
Forty days cadmium exposed and selenium supplemented fish: Testes of 40 days control fish showed no marked changes were observed in the testes when compared with the testes of 20 days control.
In the forty days cadmium exposed (10ppm) fish (fig.5). The seminiferous lobules exhibited marked degeneration of germinal elements and spermatids, spermatogonia are clumped to form a mass like structure. Selenium supplemented group (fig.6) showed disappearance of vacuolization and reappearance of chromatin material.

DISCUSSION

The present investigation iricludes the study of toxicity assessment of cadmium chloride on freshwater catfish Clarias batrachus. LC_{50} value, safe concentration and mortality, histological changes and selenium protection have been investigation. There are a few records on the effect of heavy metals on the fish tests. However some heavy metals like cadmium, copper, zinc, etc. have been reported to cause the histological changes in fish testes. Sanglang and Halioram (1974) found degeneration and necrosis of lobule boundary cells in trout testes after 28 ppm treatment with cadmium. During *in vitro* studies they also found an alteration in the steroid production in treated testes. The evidences presented here constitute a strong case for the matter that heavy metals greatly affect the testicular physiology in some fresh water teleosts. However, Sehgal et al. (1984) demonstrated the effect of copper sulphate and cadmium chloride on the testes of *L. reticulatus.* They found that both heavy metals reduced the gonosmatic index in male *L. reticulatus.* In the present investigation, when gonosomatic indices were observed it was found that cadmium exhibited more harmful effects and produced the lowest gonosomatic index in *Clarias batrachus*. These results corroborate the observations of Sehgal et al. (1984) in fish, *L. reticulatus* treated with heavy metal exposure. Selenium supplemented group of fish showed significantly increased Gono-Somatic-I ndex. Literature indicates that pollutants induce histological changes in the fish testes (Pandey and Shukla, 1980, 1982; Pandey and Sehgal, 1985).

In the present study the testes exhibited very thin and broken tubular connection tissue septa. In the centre of some lobules, spermatids and sperms seem to disappear and replaced through vacuoles. The spermatogonia spermatocytes and spermatids loose their normal structure and reveal necrosis, after 40 days of cadmium chloride (10 ppm) exposure. The present results are in conformity with the reports of Shukla and Pandey (1984a) as they found that arsenic altered the testicular activities in *Calisa fasciatus.* It has been reported that testicular damage, atrophy, haemorrhage, caused by cadmium toxicity, were protected by the simultaneous selenium injection (Yoshikawa and Ohta, 1982; Ohta, 1985). In the present investigation haemorrhage and necrosis were protected completely by the selenium (0.1 ppm) supplementation compared with those of the cadmium group when selenium cadmium complex was existed in blood and in testes (Ohta, 1985). However in this experiment cadmium concentration in testes was increasing with the disappearance of cadmium from the high molecular weight fraction in testes and blood, and almost all of the cadmium accumulated in the metallothionein fraction, which mitigated the cadmium toxicity.

The result presented here indicated that the cadmium chloride is susceptible to testes of *Clarias.* Selenium produced significant positive influence on reproductive system. In the case of selenium supplementation, cadmium was accumulated in ovary as non-toxic selenium-cadmium complexes and cadmium accumulation in ovary was delayed remarkably.

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