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Study of the effect of cadmium on the testes of the fish Puntius ticto and the cal localization of the cadmium

Rekha pundir

Department of zoology, Holkar Science College , Indore -India

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Abstract - When specimens of ***P. ticto*** were exposed dor 15 and 30 days to a safe cadmium acetate concentration , histo-physiological abnormalities were found in their testes. The changes included marked vacuolation of the cells in the seminiferous lobules and the deformation of the lobules themselves. The interstitial cells displayed necrosis . The sperm duct were thickened by fibrous tissues, so that its structure and function were affected. The gonadosomatic indexes in the treated group decreased significantly . The percentage of earlier spermatogenic cysts decreased , while the percentage of atertic apermatophore increased. Histochemical localization of calcium in the testes of ***P. ticto*** was also observed. The physiochemical parameters of the water were determined.

Key words: Fish, Cadmium testes , Physiocemical parameters.

INTRODUCTION

Cadmium, a metal highly toxic for most organism, I to be found in very low concentration (less than 5/ug/l) in natural water (kopp and kroner 1970, Lovett et al. 1972).sehgal and pandey (1984) demonstrated that lebistes reticulates was highly sensitive to cadmium under chronic exposure condition and that its testes were severelydamaged by this metal. The toxic effects of cadmium to fish has been examined extensively (spehar et, al ,1980;saxena,1986;pundir 1989,1990). The purpose of the present study was to determine the toxic effect of cadmium on the testes of the fih puntius ticto and to obtain information on the site of deposition of cadmium in chronically exposed specimens.

MATERIAL AND METHODS

Mature specimen of ***P. ticto*** weighing 325+- 2.25 mg and measuring 3.6 +/- 1.5 cm were collected from local ponds in the vicinity of Ujjain in India. For adult 15 days were acclimatized to laboratory condition and were given fish food daily. Freshly prepared cadmium acetate was diluted to the desired concentration. The safe concentration, calculated according to hartet al.(1945), was found to be 7.75 mg/l. LC 50 and LC 100 values were also calculated and were found to be 26 mg/1/96 h and 85mg/1/96 h respectively (Litchfield and wilcoxon 1949).

The fish were kept for 15 and 30 days in solution with a safe cadmium concentration, renewing the solution every three days to maintain the proper concentration for the entire duration of the experiment . the length and weight of the fish were recorded at the beginning and end of the experiment. On the 15^{th} and 30^{th} day the fish were killed and their testes were dissected out , weighed and fixed in aqueous bouin's fluid .routine paraffin Sections were prepared and stained with delafield's haematoxylin and eoin. The gonado-somatic index (GS 1) was computed (pickford 1953) , the percentual proportion of the different spermatogenic stages wa recorded and the physiochemical parameters of the water were also studied (APHA 1975)
Cadmium was demonstratred in the tissues by the silver sulphide method (pearse 1972) . the tissues were fixed 8 h in 10 % ethano aturated with H2S and alkanised by the addition of two drops of concentrated ammonia per 100 ml. after fixation the tisues were dehydrated in alcohol , cleared in xylene , embedded in paraffin and was sectioned at 4-5/um . the sections were treated 2 h with developer reagent , in the dark , at 22 degree C ; they were then washed in distilled water, counterstained 2 min with saffarin and mounted in DPX. The heavy metal salt showed up in brownish black against a red background. The control and experimental sections were compared.

OBSERVATIONS

The physiochemical parameters of the water are given in tab 1.
The testes of ***P. ticto*** is a bean shaped structure composed of radially organized lobules , whose coelomic surface is bounded by thin visceral peritoneum. The basal laminae of adjacent lobules are separated by interglobular septa . the seminiferous lobules are lined with germinal epithelium. The section, each lobule is seen to contain germ cells at various stages of differentiation (spermatogenesis, spermatocytes, spermatids, spermatozoa).the interstitial cells appear to communicate with each other via interlobular connective

tissue . the narrow sperm duct is ined with distinct simple squamous epithelium. (fig 1, 2)

Fifteen days treatment with a safe dose od cadmium ed to drastic changes in the tetes of ***P. ticto***. The seminiferous lobules were severely affected and displayed structural abnormalities. Vacuolation and degeneration of the germ cells were observed. The wall of the lobules disintegrated, exposing their contents. The connective tissue showed destruction of septal demarcation in the treated group. The germinal core of the lobules comprised degenerating cells , most of which atrophied; their cytoplasm was liquefied and their nuclei exhibited degerative changes. Necrotic changes took place in both the cytoplasm and the nuclei of the spermatogonia. The primary and secondary spermatocytes were distinctly vacuolated and the spermatids also displayed deformities associated with degeneration of their nuclear material and vacuole formation (Fig. 3,4) in their cytoplasm. The sperm cysts no longer had a compact appearance. The interstitial cells were atrophied. The percentage of atretic sperm cycts (spermatophores) increased and the percentange of the other types of spermatogenic cysts decreased. The gonado-somatic index fell compared with the controls (Tab.II) . In treated fish,fine cadmium sulphide granules appered in the testes . the wall of the spermatocytes and spermatozoa had a dark cadmium sulphide band (Fig. 5). A few fine metal granules also appeared in the interstitial cells.

Thirty days' treatment with a safe concentration of the heavy metal cadmium produced striking changes in the testes of ***P. ticto***. They showed clear reduction of testicular activity , as reflected in a significantly lower gonado-somatic index compared with the control (Tab II). Complex changes were recorded , such as reduction of the size of the testes and striking shrinkage of the interstitial cells in the treated group . The seminiferous lobules showed irregular arrangement patterns. The spermatogonia became highly vacuolated and hyperplastic. In the primary and secondary spermatocytes, the damage involved degeneration of the nuclear material and vacuolation of the cytoplasm (Fig. 6). Large spaces were observed between the lobules. The sperm duct exhibited irregularity of structure and the spermatozoa inside the duct showed deformities. The interstitial cells atrophied. Ruptured connection tissue septa were seen in the treated group. The sperm cysts underwent atresia. The percentage of atretic sperm cysts increased and the percentage of the other spermatogenetic stages decreased (tab. II).

DISCUSSION

In this study , therefore, spermatogenesis in ***P.ticto***. was impaired by exposure to cadmium, with deformities of the spermatogonia , spermatocytes , spermatids and spern cysts after 15 and 30 days' exposure. The effects after 30 days were more pronounced. The interstitial cells atrophied. These results are in agreement with the reports of Shukla and Pandey (1984) and Pundir (1990 & 1993) , who found that the heavy metal altered testicular activity in fishes . there is no previous record of the deposition of cadmium in teleostean testes. In the present investigation. Cadmium granules were localized in the spermatocytes , spermatids and sperm cysts.

It is evident from the above results that spermatogenesis in ***P. ticto***. was partly inhibited by cadmium treatment. It is difficult to find a satisfactory explanation of how the heavy metal affects the pituitary-testes relationship in fish. The most plausible suggestion is that secretion of the gonado-trophins which regulate testicular activity is impaired.

Table I.
Physico-chemical parameters of control and cadmium treated water (mean values)

Characteristics	Control Water	Cadmium treated Water
Temperature	20.0	20.0
Total Alkalinity	30.5	39.9
Total hardness	75.1	110.2
pH	8.2	8.4
Dissolved Oxygen	3.5	2.8
Biological Oxygen	4.5	5.5
Chemical Oxygen demand	35.1	40.1
Free Chlorine	1.0	1.0
Free Carbon-di-oxide	0	0

All values are expressed in mg/l except pH and temperature.

Table II.
Gonadosomatic index (GSI) and percentage of spermatogenetic stages in *P.ticto*. (Mean Values)

Parameters	Control	Cadmium treated group	
		15 days	30 days
GSI	12.46	5.67*	4.64*
Spermatogonia	12.1	10.0**	8.5
Primary Spermatocytes	20.1	18.1**	13.5*
Secondary Spermatocytes	20.2	18.1**	14.1*
Spermatids	22.4	20.1**	16.5*
Sperms	17.1	16.1	12.1*
Atretic sperms cysts	7.0	17.2*	28.2*

*P < 0.001 , **P < 0.01

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Explanation of figures

Fig. 1-7. Sagittal sections of testes of *P.ticto*.

Fig.1.

Control testis showing seminiferous lobules (SL) , spermatogonia (SG) and primary spermatocytes (PSC). x 1,000.

Fig.2.

Control testis showing secondary spermatocyte (SSC) and interstitial cells (IC). x 1,000.

Fig.3.

Section of testis of fish treated 15 days with cadmium, showing vacuolation of the spermatocytes and atrophied interstitial cells (IC). x 1,000.

Fig.4.

Section of testis of fish treated 15 days with cadmium, showing vacuolation of germ cells and disorganization of the semiferous lobules (SL). x 600.

Fig.5.

Section of testis of fish treated 15 days with cadmium, showing deposition of cadmium in the seminiferous lobules (SL) and vacuolation of the germ cells. x 1,000.

Fig.6.

Section of testis of fish treated 30 days with cadmium, showing vacuolation of spermatogonia (SG), primary (PSC) and secondary (SSC) spermatocytes and spermatids (ST) and atrophy of interstitial cells (IC). x 1,000.

Fig.7.

Section of testis of fish treated 30 days with cadmium , showing deposition of cadmium in the seminiferous lobules(SL). x 600.

Abbreviations : CS- cadmium , IC- interstitial cells, PSC- primary spermatocytes , S- spermatozoa , SG- spermatogonia , SL- seminiferous lobules , SSC- secondary spermatocytes , ST- spermatids , VC- vacuoles.