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Research Paper

Effect of Cadmium chloride on ovarian histology of *Heteropnuestes fossilis*

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Abstract

The present study was carried out to evaluate the sub lethal effect of cadmium chloride in the ovaries of Heteropnuestes *fossils* ,which were kept in aqueous solution of cadmium chloride i.e.(0.1 ppm) for duration of 7, 14, 21 days. The histological effect of cadmium chloride in ovaries is characterized by enlarged oocytes, degeneration of outer layer of oocytes, and ruptured ovarian follicle interafollicular space exhibited in all groups.

Keywords: Cadmium chloride, fish, heavy metal, interfollicular space, ovary

Introduction

Histopathology is promising field for research in aquatic toxicity as it provides the real picture of the toxic xenobiotic in vital function of a living organism [1]. Cadmium is naturally released in to the environment from volcanic sources and along with rain water to reaches up to the water bodies [4]. It act as an endocrine disrupter interfering with biological function such as reproduction , growth , development, osmoregulation and the ability to cope up with stress in fish[11]. Cadmium has been shown to be responsible for a number of reproductive abnormalities in fish [12]. It has been found that cadmium is generally accumulated in major organ of fish like ovary and testis [7]. Heavy metal also affected sexual differentiation of the gonads timing of sexual maturation gonodosomatic index, reproductive tract and gonad morphology [9].

Vitellogenesis has been shown to be altered in winter flounder due to cadmium contamination [10]. Cadmium is able to delay ovulation or to disrupt vitellogenesis in trout [5]. The impact of cadmium on the ovaries of female fish causes their delayed or no morphosis and interference with their gonad development [6]. The present work therefore designed to study the effect of cadmium chloride at safe concentration in the oogenetic stages of ovary of fish H. fossilis.

Materials and Methods

Experimental Animal

Living and healthy species of H. fossilis (wt. 20±3g, L. 13±3cm.) were collected from local fisherman of Ujjain and were kept in glass aquaria and acclimatized to the laboratory condition for 7 days and washed with Kmno4 solution to avoid dermal infections. They were fed chopped and dried prawn daily and water renewed an alternate day.

Toxicant- 0.1ppm cadmium chloride solution was used for present experiment.

Experimental design

Fishes were divided into groups control and treated. Seven fish in each group were kept in glass aquaria. Each containing 20 liter water no mortality was recorded during experimental period. The fishes of experimental and control group were dissected in 7, 14 and 21 days for histological study.

The ovary was taken from control and treated group of fishes were fixed in Bouin´s solution. The material was dehydrating through graded alcohol. After filtration in paraffin wax (58 C) for 3hr. Block were prepared and stretched and stain with H&E stain. Microscopic observation is made after staining process. The gonosomatic index was calculated after formula given by Pickford:

$$GSI = \frac{\text{weight of ovary}}{\text{Total weight of fish}} \times 100$$

The randome sampling (20 each) was done to determine the number and diameter of different oocytes in the ovary. The mean diffrence was determined by mean± SE and compared with Student "t" test.

Results and Discussion

Control Group

The ovary of this fish is paired and attached to the dorsal wall of the body cavity. Two ovaries close to each other. The wall of the ovary consists of a single layer of germinal epithelium which spheres the tunica albuginia. Different stages of different types of oocytes were observed in control group. The oogonia are smallest in size and arranged in follicular lamillae. The immature oocytes are same what bigger in size and have evenly distributed cytoplasm while immaturing oocytes are in prenucleolar and yolk vesicle stage they have well defined structure. The oocyte of perinucliolor stage were rounded in the yolh vesicles appeared in the cytoplasm of yolk vesicles stage oocytes were seen with thin follicular layer and nucleoli were on nuclear membrane. The mature oocytes are big in size and surrounded by theca externa, interna and granulosa cells.they encirde the yolk globules. The strong reaction with Heamatoxyline Eosin was seen in all oocytes (Fig.1, 2, 3).

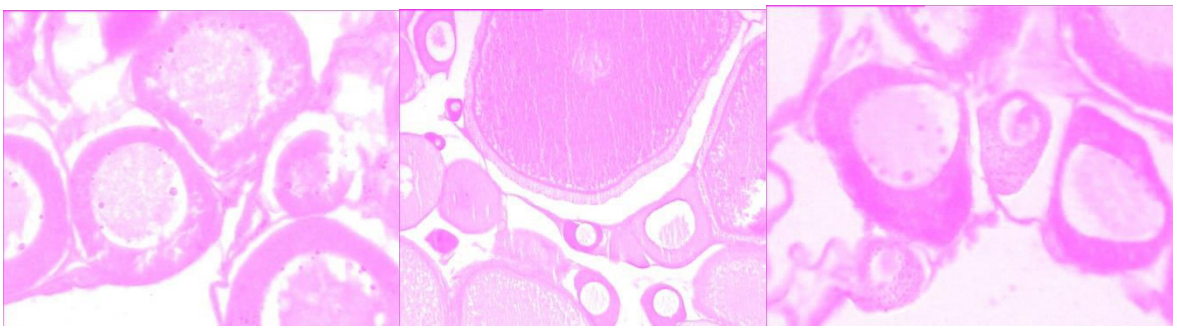


Figure 1 **Figure 2** **Figure 3**

Figure 1: **CONTROL GROUP (7DAYS): Showing the normal histological structure of ovary different type of oocytes was observed (HE x 40).**

Figure 2: **CONTROL GROUP (14 DAYS): Oogonia clearly seen and distributed in cytoplasm. The oocyte of perinucliolor stage were rounded in shape and showed enlarge nucleus (HEx40).**

Figure 3: **CONTROL GROUP (21 DAYS): Section showing the normal histological structure of ovary. Nucleus was clearly seen large spherical and smooth in the middle of oocytes (HEx40).**

Treated Group

After 7 day exposure of cadmium chloride tunica albugenea were become thick and ruptured place. Oogonia were surrounded by deformed epithilial cells. Maturing oocytes was disrupted and in perinucleolar yolk vesicles stage. The cytoplasm were degenerated and deformation in cytoplasm and nuclear content were prominent in while perinucleolar stage they were large and deformed in shape. The mature oocytes had deformed shapes with comperision to control. The wide spaces exhibited in treated granule due to loss of structural tissue in mature oocytes (Figure 4).

In 14 days exposure loss of normal configuration of primary oocytes, necrosis, elongated ovarian follicles and fragmented cytoplasm with abnormal shape. In immature oocytes nuclear contents were showed necrotic condition. The theca and granulosa layer exhibit dissolution, while the yolk granules become condensed. The atretic oocytes were also seen in this duration (Figure 5).

In 21 day duration of treatment the outer tunica albuginia layer become fibrous and wavy Due to accumulation of fibrous tissue the layer become thick with comperision to control. The oogonia exhibit thick and necrotic configuration while the immaturing oocytes lost its cytoplasmic content and become granule the maturing oocytes exhibit,thick germinal epithilial lining and become fibrous. The granulation of cytoplasm content was marked. The loss of nuclear content was also evident. The mature oocytes were in deformed condition. The outer theca and internal layer become disintegrated and had liquified appearance. The granulosa layer was shrinked and encircled deformed yolk granules. Due to this wide gape exhibited between the theca and granulosa layer. At place these layer were interrupted due to cadmium load (Figure 6). In all these duration the gonosomatic index ($p<0.01$) reduced (Table 1) and the diameter was reduced with comparison to control (Table 2, 3, 4).

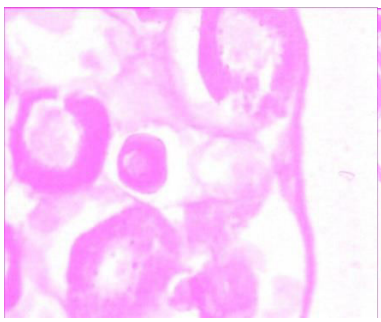


Figure 4

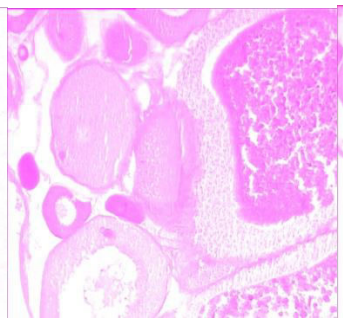
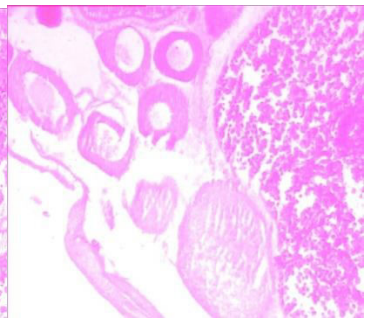


Figure 5



Figures 6

Figure 4: CADMIUM CHLORIDE TREATED GROUP (7 DAYS): Showing the deformed oogonia in primary oocytes. Maturing oocytes had deformed cytoplsme and nuclear mater. Tunica albugenea were become thick at ruptured place. (HE x 40)

Figure 5: CADMIUM CHLORIDE TREATED GROUP (14 DAYS): In primary oocytes nuclear contents were showed necrotic condition. The atretic oocytes were also seen in this duration (HE x 40)

Figure 6: CADMIUM CHLORIDE TREATED GROUP (21 DAYS): The oogonia exhibit thick and necrotic while the immature oocytes lost its cytoplasmic content and become granule the immature oocytes exhibit, thick germinal epithelial lining and become fibrous. Place these layer were interrupted due to cadmium load (HE x 40)

Table 1: GSI in Different groups of female fish, Heteropneustes fossilis

S. No.	No. of days	Control Group	treated Group
1	7	6.5 ± 0.07	4.4 ± 0.69 *
2	14	9.6 ± 0.54	7.6 ± 0.64 *
3	21	10.9 ± 0.64	7.3 ± 0.19 *

All value is expressed in Mean±SEM, Total no. of samples: 6, Significance level: (* $P<0.05$, ** $P<0.01$)

Table 2: Diameter of oogenetic stages in different experimental group of *H. fossilis* (7Days)

S. No.	Stages of Oocytes	Diameter of Oocytes (in mm)	
		Control group	cadmium chloride Treated group
1	Peritoneal covering	0.015±0.001	0.009±0.001 ***
2	Oogonia	0.007±0.0004	0.005±0.0004 **
3	Immature	0.03±0.001	0.01±0.001 **
4	Maturing	0.04±0.001	0.02±0.001 **
5	Mature	1.0±0.001	0.50±0.001 **

All values are expressed in Mean± SEM; Total no. of samples for each observation: 20 Significant levels (* P<0.05, ** P< 0.01, **P<0.001)

Table 3: Diameter of Oogenetic stages in different experimental group of *H. fossilis* (14 Days)

S. No.	Stages of Oocytes	Diameter of Oocytes (in mm)	
		Control group	cadmium chloride Treated group
1	peritoneal covering	0.0115±0.001	0.002±0.0002 *
2	Oogonia	0.009±0.0002	0.008±0.0004 *
3	immature	0.05±0.001	0.02±0.001 **
4	Maturing	0.06±0.001	0.03±0.001 **
5	mature	1.10±0.001	0.80±0.001 **

All values are expressed in Mean± SEM; Total no. of samples for each observation: 20 Significant level (* P<0.05, ** P< 0.01, *** P< 0.001)

Table 4: Diameter of Oogenetic stages in different experimental group of *H. fossilis* (21days)

S. No.	Stages of Oocytes	Diameter of Oocytes (in mm)	
		Control group	Cadmium chloride treated group
1	Peritoneal covering	0.0100±0.001	0.0043±0.0004 *
2	Oogonia	0.006±0.0003	0.004±0.0004 ***
3	Immature	0.08±0.001	0.05±0.001 **
4	Maturing	0.9±0.001	0.3±0.001 **
5	Mature	2.10±0.001	1.80±0.001 **

All values are expressed in Mean± SEM; Total no. of samples for each observation: 20 Significant level (* P<0.05, ** P< 0.01, *** P< 0.001)

Endocrine system plays an important role in the control of physiological processes, reproduction, metabolism and growth of fish[2]. Cadmium has been shown to be responsible for a number of reproductive abnormalities in fish[12]. The ovary of fish *Cyprinus carpio* exposed to 0.5 ppm mercuric cloride exhibited large degenerative change with decreased gonosomatic index [8]. Baruah and Das also noted partial lysis, swelling atresia and change in ovarian nucleus after Cd exposure for 20 days. Long term exposure of cadmium chloride in *H. fossilis* resulted in marked degenerative changes in the ovary[3]. These changes included prominent interfollicular space, appearance of atretic follicles, and degeneration in nucleus degenerative in the ovarian follicles[12]. In our present work study the cadmium chloride affects normal structure of all oogenetic stages of the ovary of *H. fossilis*. This may be due to accumulation of cadmium the developing oocytes or due to stress. The different oogenetic stages were arrested and become necrotic.

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