E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2018; 6(2): 601-605

www.fisheriesjournal.com
Received: 03-01-2018
Accepted: 04-02-2018

Nirmal Singh Sengar
School of Studies in Zoology & Biotechnology, Vikram University Ujjain, Madhya Pradesh, India

Lata Bhattacharya
School of Studies in Zoology & Biotechnology, Vikram University Ujjain, Madhya Pradesh, India

Ankita Soni
College of life sciences, Gwalior, Madhya Pradesh, India

Alok Kumar Soni
National Institute of Pharmaceutical Education and Research, Mohali, Punjab, India

Correspondence
Alok Kumar Soni
National Institute of Pharmaceutical Education and Research, Mohali, Punjab, India

Ameliorating effect of mulethi herb in cadmium chloride induced toxicity in ovary of *Heteropnuestes fossilis* fish

Nirmal Singh Sengar, Lata Bhattacharya, Ankita Soni and Alok Kumar Soni

Abstract
The present study was carried out to evaluate the sub lethal effect of cadmium chloride in the ovaries of *Heteropnuestes fossils* fish and protective effect of Mulethi herb. The Fishes were divided in to three group, first as a control, second group was cadmium treated group in which fishes exposed to 0.1 ppm concentration of cadmium chloride ($cdcl_2$) in water for 7, 14 and 21 days. Third group were exposed with $cdcl_2$ and treated with Mulethi extract mixed with fish food pellet. We observed cadmium toxicity in fish reflecting by gonadosomatic index (GSI) and histological alteration. Cadmium exposure caused enlarged oocytes, degeneration of outer layer of oocytes also ruptured ovarian follicle and increased interafollicular space. Moreover herbal treatment of Mulethi extract was able to recover and improve these alterations and protect fish from cadmium toxicity.

Keywords: cadmium chloride, fish, heavy metal, ovary, mulethi

1. Introduction
Aquatic ecosystems are major recipients of pollutants. These major pollutants prevailing in environment are heavy metals, pesticides, synthetic polymers etc. Industrial discharges containing toxic and hazardous substances including heavy metals such as arsenic, cadmium and they contribute tremendously to the pollution of aquatic ecosystems [1]. Heavy metals adversely affect aquatic fauna and flora and their presence cause contamination in aquatic ecosystem that poses a serious environmental hazard [2]. The major problem of heavy metal toxicity is the persistence and tendency to accumulate in organisms. The toxicity of heavy metals has been reported in vital organ of aquatic animals caused by oxidative stress and inflammation [3]. Moreover heavy metal also affects sexual differentiation of the gonads and timing of sexual maturation, reproductive tract and gonad morphology [4]. Cadmium is naturally released in to the environment from natural as well man made source and progressively reach to the water bodies along with rain water and canals. Fish are considered as one of the primary risk organism for toxicity study and predominantly endocrine disrupting chemicals including heavy metals [5].

Fishes are found virtually everywhere in the aquatic environment and they play a major ecological role in aquatic food webs because of their function as carrier of energy from lower to higher tropic levels. Cadmium has been shown to be responsible for a number of reproductive abnormalities in fish [5]. 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 [6]. Moreover it has been found that, cadmium is generally accumulated in major organ of fish like liver, kidney and reproductive organ such as ovary and testis [7]. The impact of cadmium on the ovaries of female fish causes their delayed or no morphosis and interference with their gonad development [8]. The treatment of heavy metal toxicity is chelation therapy, which promote the excretion of these metals. The compound used in chelation therapy are, dimercaprol, dimercaprosuccinic acid and EDTA [9]. However some naturally herb and other dietary supplement may help in reducing oxidative stress and maintain nutrients sufficiency. In previous reports also, Mulethi (*glycirhiza glabra*) an herb, which has been found to be protective in metal toxicity in vital organ such as kidney and testis [10, 11]. 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* and recovery of tissue by Mulethi.

2. Materials and Methods

2.1 Experimental Animal

Living and healthy species of *H. fossilis* (weight range 18-22 gram and Length 10-12 cm.) were collected from local fisherman of Ujjain. Fishes were kept in glass aquaria and acclimatized to the laboratory condition for 7 days and also washed with $KMnO_4$ solution to avoid dermal infections. They were fed with standard chopped and dried prawn daily and water renewed on alternate day.

2.2 Experimental design

Fishes were divided into three group's control, cadmium treated and recovery (cadmium and Mulethi treated) groups. Seven fish in each group were kept in glass aquaria. Each tank containing 20 liter water and 0.1 ppm concentration of cadmium chloride was used for present experiment. In recovery group Mulethi extract were mixed with food pellet. The fishes were dissected in 7, 14 and 21 days.

2.3 Histopathological study

On 7, 14 and 21 days, fishes from different groups were dissected and ovary was remove and processed as follows: the ovary tissues were washed in cold saline then fixed with Bouin´s solution. Samples were embedded in paraffin, then sectioned (5 µm) using a microtome stained with hematoxylin and eosin (H&E), and examined with light microscopy.

2.4 Gonadosomatic index

Gonadosomatic index (GSI) is a metric that represents the ratio between gonad weight (here we took ovary) and body weight of fish. In each individual sampled, the Gonadosomatic index was calculated using the Pickford formula [12].

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

The random sampling (20 each) was done to determine the number and diameter of different oocytes in the ovary. The data were analyzed using one way analysis of variance and Student t test was done for comparison between groups. $P<0.05$ was considered statistically significant. The analyses were carried out using sigma stat software version 2.03 (SPSS, USA).

3. Results

Histopathology change

1. Control group

Histopathology of fishes from control group was done for 7, 14 and 21 days. Results suggested that 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. Histopathological results suggested that, oogonia were smallest in size and arranged in follicular lamellae. The immature oocytes were bigger in size and also their cytoplasm was evenly distributed with thin follicular layer and nucleoli and nuclear membrane. While in immature oocytes, cytoplasm was perinucleolar and yolk vesicle stage with well defined structure. The mature oocytes were bigger in size and surrounded by theca externa, interna and granulosa cells. (Fig.1).

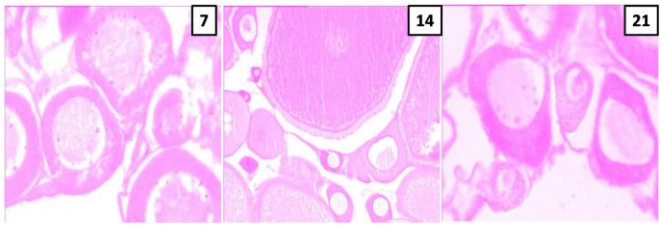

Fig 1: Histopathological results of fishes from Control group (A: 7 day): Showing the normal histological structure of ovary different type of oocytes was observed (B: 14 day): Oogonia clearly seen and distributed in cytoplasm. The oocytes of perinucleolar stage were rounded in shape and showed enlarge nucleus. (C: 21 day): Section showing the normal histological structure of ovary. Nucleus was clearly seen large spherical and smooth in the middle of oocytes (HEx40)

2. Cadmium treated group

In present study, no mortality was recorded during experimental period. And LC50 value of cadmium chloride was found to be 0.5 ppm for 72 hrs in *H. fossilis*. Histopathology of Cd treated group revealed that, after 7day treatment of cadmium chloride, the tunica albugenea were became thick and ruptured place. Oogonia were surrounded by deformed epithelial cells. Maturing oocytes were disrupted and in perinucleolar yolk vesicles stage. The cytoplasm was degenerated and deformed and nuclear content were perinucleolar, large and were deformed in shape. The mature oocytes had deformed shapes with comparison to control. The wide spaces exhibited in treated granule due to loss of structural tissue in mature oocytes (Fig.2).

In 14 days treated group there was loss of normal configuration of primary oocytes, necrosis and elongated ovarian follicles was observed. Apart from that, these oocytes were showing fragmented cytoplasm with abnormal shape. In immature oocytes nuclear contents were showed necrotic condition. The theca and granulosa layer was in quite dissolution state, while the yolk granules became condensed. The atretic oocytes were also seen in this duration (Fig.2).

After 21 day duration of cadmium treatment, the outer tunica albuginia layer became fibrous and wavy due to accumulation of fibrotic tissue. We also found cadmium exposure leads to oogonial layer thick with compared to control and also showed necrotic configuration. Apart from that, the immature oocytes were lost its cytoplasmic normal content and became granular in nature. Moreover the histopathological analysis of maturing oocytes showed, thick germinal epithelial lining and become fibrous. The granulation of cytoplasm content was marked. The loss of nuclear content and deformation was also observed after 3 week of cadmium treatment evident. The mature oocytes were in deformed condition. The outer theca and interna layer become disintegrated and had liquefied appearance. The granulosa layer was shrieked and encircled deformed yolk granules due to wide gape exhibited between the theca and granulosa layer (Fig.2). In all these duration the Gonadosomatic index reduced significantly ($p<0.01$, Table 1) and the diameter of ovary was reduced with comparison to control (Table. 2, 3, 4).

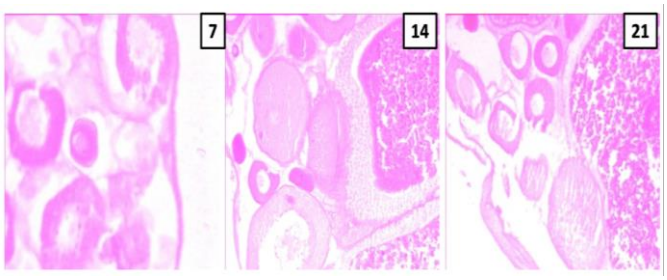

Fig 2: Histopathology of cadmium chloride treated group (7 days): Showing the deformed oogonia in primary oocytes. Maturing oocytes had deformed cytoplasm and nuclear mater. Tunica albugenea were become thick at ruptured place. (14 day): In primary oocytes nuclear contents were showed necrotic condition. The atretic oocytes were also seen in this duration (21 day): 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)

3. Recovery group

In the recovery group of Mulethi, in 7 days the ovarian membrane occupied its normal position. The oogonial germ cells were visible beneath the surrounding layer of ovary. Moreover chromatin content in oocytes (nucleolar and perinucleolar) showed normal architecture compared with cadmium treated group. The oocytes of perinucleolar stage exhibited increased volume of cytoplasm and regenerated nuclear contents. A few atretic oocytes were also seen in this duration. The interfollicular spaces were reduced than that of treated group and oocytes of yolk globule stage exhibited regeneration of granulosa layer and yolk globules were evenly spreaded below this layer. Reformed shape of different oocytes was more prominent after one week of mulethi treatment. (Fig.3)

In 14 days recovery of mulethi the ovarian membrane was reformed at some places in the ovary of mulethi recovery group. The oogonial nests were seen in this duration and their populations were dramatically increased. Reformed follicular layer was observed in oocytes of yolk vesicle stage with normal ooplasm and full of yolk vesicles. The surrounding layer of yolk granule stage was became thin and reappeared yolk granules were seen in maturing oocytes (Fig.3)

The histopathology analysis of fish's ovary with 21 days treatment of mulethi in recovery group revealed, reformed and intact ovarian membrane. The primary oocytes were reformed in their shape and interfollicular spaces were greatly reduced. In some case residual oogonia were observed in this duration. Their nuclear contents were regenerated and cytoplasm was liquefied. Apart from that the oocytes of perinucleolar stage showed rearranged nucleoli at the periphery of nucleus. The yolk vesicles reappeared in the ooplasm of yolk vesicle stage and similarly yolk globules also returned into normal in the cytoplasm of yolk globule stage. Regenerated granular texture was more prominent in maturing oocytes than treated group. These yolk granule and mature oocytes showed clearly distinguished theca layer, zona granulosa and zona radiata. Nucleus was spherical in shape and clearly visible in vitellogenic oocytes. Mature oocytes attained their normal architecture compare to ovary of cadmium treated group. Moreover they were packed with yolk granules as seen in control group (Fig.3)

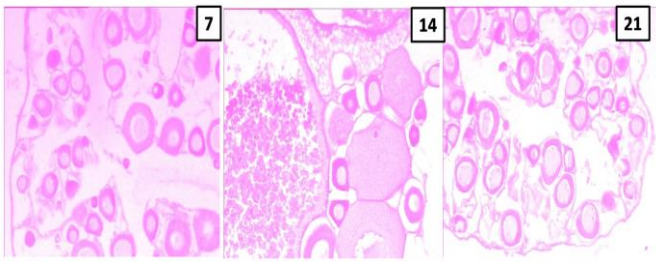

Fig 3: Mulethi recovery group (7 day): showing increased oogonia germ cells attached to ovarian membrane, improved staining affinity of ooplasm in different oocytes and deshaped perinucleolar stage along with few atretic oocytes (14 Day): showing regenerated cytoplasm in primary oocytes, many yolk vesicles inside the yolk vesicle stage, wide spaces among the oocytes still persistent and atretic masses were also visible. (21 day): showing regenerated cellular contents and reappearance of theca and granulosa layer and increased compactness. (HE x 40)

Apart from histological results, the mean GSI value of female fish of control group was 6.5±0.71, 9.6±0.54 and 10.9±0.64 for 7, 14 and 21 days respectively. After the treatment of cadmium chloride, it was significantly ($P<0.05$) decreased for 7 and 14 days treated group and ($P<0.01$) decreased for 21 days group. In recovery groups of Mulethi, it was significantly ($P<0.05$) increased in 7, 14 and 21 days duration than that of compared treated groups.

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

S.No.	No. of days	Control Group	Cadmium chloride treated Group	Recovery Group Mulethi
1	7	6.5 ± 0.71	4.4 ± 0.69 *	6.4 ± 0.38†
2	14	9.6 ± 0.54	7.6 ± 0.64 *	9.4 ± 0.47 †
3	21	10.9 ±0.64	7.3 ± 0.19 *	9.31 ± 0.51†

All value is expressed in Mean±SEM, Total no. of samples: 6. *p<0.05 when compared with normal control group; †p<0.05 when compared with cadmium treated group.

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	Recovery group
1	Peritoneal covering	0.015±0.001	0.009±0.001 *	0.010±0.001†
2	Oogonia	0.007±0.0004	0.005±0.0004 *	0.007±0.0001†
3	Immature	0.03±0.001	0.01±0.01 *	0.02±0.001†
4	Maturing	0.04±0.001	0.02±0.05 *	0.03±0.001†
5	Mature	1.0±0.001	0.50±0.01 *	0.90±0.001†

All values are expressed in Mean± SEM; Total no. of samples for each observation: 20. *p<0.05 when compared with normal control group; †p<0.05 when compared with cadmium treated group.

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	Recovery group
1	Peritoneal covering	0.0115±0.001	0.002±0.0002 *	0.0101±0.0002†
2	Oogonia	0.009±0.0002	0.008±0.0004 *	0.01±0.007†
3	immature	0.05±0.001	0.02±0.01 *	0.04±0.001†
4	Maturing	0.06±0.001	0.03±0.02 *	0.06±0.001†
5	Mature	1.10±0.001	0.80±0.01 *	1.10±0.001†

All values are expressed in Mean± SEM; Total no. of samples for each observation: 20.*p<0.05 when compared with normal control group; †p<0.05 when compared with cadmium treated group.

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	Recovery group
1	Peritoneal covering	0.0100±0.001	0.0043±0.0004 *	0.0066±0.0001
2	Oogonia	0.006±0.0003	0.004±0.0004 *	0.005±0.0004†
3	Immature	0.08±0.001	0.05±0.05 *	0.06±0.001†
4	Maturing	0.9±0.001	0.3±0.01 *	0.6±0.001†
5	Mature	2.10±0.001	1.80±0.03 *	1.80±0.001†

All values are expressed in Mean± SEM; Total no. of samples for each observation: 20 Significant level *p<0.05 when compared with normal control group; †p<0.05 when compared with cadmium treated group.

4. Discussion

Heavy metals such as Cadmium considered as one the most toxic environmental pollutant. It is nonessential element to all living organism. Aquatic lives are the primarily affected area by diluted cadmium waste industries [9]. It interferes with biological functions such as reproduction, growth, development, osmoregulation and the ability to cope up with stress in fish. It causes significant metabolic alteration and injuries of biological system at different levels [10]. An endocrine system particularly steroid hormone plays an important role in the control of physiological processes, reproduction, metabolism and growth of fish. Cadmium has been shown to be responsible for a number of reproductive abnormalities in fish and hormone [13]. Sub lethal effect of cadmium also includes alteration in metabolism and appetite of fish resulting in less active and less food intake. As fish age, heavy metal and metallothionein concentration in vital organ, increase under chronic exposure which causes serious effect [14]. Cadmium is an endocrine disrupter and it inhibits hormone synthesis and ovarian maturation [15]. The gonadosomatic index is a very good indicator of the state of gonadal development and many times it has been taken into account for the estimation of reproductive toxicity of heavy metal in fish [16]. In one such study of heavy metal mercury caused reproductive deformation and gonadosomatic alteration. In this study fish *Cyprinus carpio* were exposed to 0.5 ppm of mercuric chloride results suggested that ovary was dramatically affected by mercury which was characterized by large degenerative change with decreased gonadosomatic index [16]. In present study we exposed fish to 0.1 ppm of cadmium chloride and we found that cadmium caused deformation in oocytes and even interfollicular space and it was gradually increased from first to third week as exposure days increased. However in mulethi treated group these histological alterations dramatically improved. Research suggested that heavy metals may cause deleterious effects on fish reproductive system. It is also suggested that heavy metal concentrations usually accumulates very high in internal organs such as reproductive tissue when compared to muscle tissues. They may interfere with gamete formation and development due to endocrine disruption and reduced hormone synthesis. In another investigation researcher has found that toxicity by cadmium after 20 days of exposure includes partial lysis, swelling atresia and change in ovarian nucleus [17]. So, present study explored that long term exposure of cadmium chloride in *H. fossilis*, resulted in marked degenerative changes in the ovary. These studies favor our finding in sub lethal dose and histopathology as well as GSI index support present study. These changes included prominent interfollicular space, appearance of atretic follicles, and degeneration in nucleus degenerative in the ovarian follicles.

5. Conclusion

In present study the cadmium chloride caused abnormal structure in all oogenetic stages of the ovary of *H.fossilis*. Histopathology observation and gonadosomatic index suggested that cadmium toxicity caused endocrine disruption in which, different oogenetic stages were arrested and they became necrotic. This may be due to accumulation of cadmium into developing oocytes or due to cadmium induced oxidative stress and inflammation Mulethi showed effective role in recovering the damage. The result of proposed study will add new information related to effective role of natural herbal treatment with Mulethi in fish physiology against cadmium chloride.

6. Acknowledgement

The author is deeply thankful to the Professor and Head DR. M.S. Parihar S.S in Zoology and Biotechnology, Vikram University Ujjain (Madhya Pradesh) for providing necessary facilities during the research work. Nirmal Singh Sengar is thanks to university grant commission (UGC) for providing financial support.

7. References

1. Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ. Heavy metal toxicity and the environment. Molecular, Clinical and Environmental Toxicology. 2012; 133-164.
2. Vinodhini R, Narayanan M. Bioaccumulation of heavy metals in organs of fresh water fish *Cyprinus carpio* (Common carp). International Journal of Environmental Science & Technology. 2008; 5(2):179-82.
3. Pourang N. Heavy metal bioaccumulation in different tissues of two fish species with regards to their feeding habits and trophic levels. Environmental Monitoring and

Assessment. 1995; 35(3):207-19.
4. Ankle GT, Giesy JP. Endocrine disruptors in wildlife a weight of evidence perspective. In Kendall R, Dickerson R, Suk W, Giesy JP, eds. Principles and Processes for Assessing Endocrine Disruption in Wildlife. Pensacola SETAC Press. 1998, 349-368.
5. Flick DF, Kraybill HF, Dlmitroff JM. Toxic effects of cadmium: a review. Environmental Research. 1971; 4(2):71-85.
6. Larsson Å, Haux C, Sjöbeck ML. Fish physiology and metal pollution: results and experiences from laboratory and field studies. Ecotoxicology and Environmental Safety. 1985; 9(3):250-81.
7. Thomas P. Teleost model for studying the effects of chemicals on female reproductive endocrine function. Journal of Experimental Zoology Part A: Ecological Genetics and Physiology. 1990; 256(S4):126-8.
8. Brown V, Shurben D, Miller W, Crane M. Cadmium toxicity to rainbow trout *Oncorhynchus mykiss* Walbaum and brown trout *Salmo trutta* L. over extended exposure periods. Ecotoxicol and Environmental Safety. 1994; 29:38-46.
9. US Environmental Protection Agency (EPA). Cadmium compounds hazard summery created in April 1992; revised in January 2000. Accessed March 8, 2018 http://www.epa.gov/ttn/atw/hlthef/cadmium.html.
10. Flora SJ, Mittal M, Mehta A. Heavy metal induced oxidative stress & its possible reversal by chelation therapy. Indian Journal of Medical Research. 2008; 1(4):501.
11. Nagar B, Bhattacharya L. Effect of cadmium chloride on kidney of *Heteropneustes fossilis* and their recovery by herbal compound Mulethi. International Journal of Research and Biosciences. 2016; 5(4):36-42.
12. Mukati K, Bhattacharya L. Effect of Cadmium Chloride on Testis of *Heteropneustes fossilis* and Recovery by Herbal Compound Mulethi. International Journal of green and herbal chemistry. 2017; 6(3):86-95.
13. Pickford GE, Atz JW. The physiology of the pituitary gland of fishes. New York Zoological Society, 1957.
14. Okocha RC, Adedeji OB. Overview of cadmium toxicity in fish. Journal of Applied Sciences Research. 2011; 7(7):1195-207.
15. Kime DE. Endocrine disruption in fish. Springer Science & Business Media. 2012, 6.
16. Masud S, Singh IJ, Ram RN. First maturity and related changes in female *Cyprinus carpio* in response to long term exposure to a mercurial compound. Journal of Ecophysiology and Occupational Health. 2003, 3-14.
17. Sharma S, Manhor S, Qureshi TA, Kaur P, Dar BA. Histological Studies on the Cadmium Chloride Exposed Air-Breathing Fish, *Heteropnuestes fossilis* (Bloch) With Special Reference to Ovaries. International Journal of Environmental Sciences. 2011; 2(2):411.