Journal of Global Biosciences
ISSN 2320-1355
Volume 4, Number 8, 2015, pp. 3037-3043
Website: www.mutagens.co.in
E-mail: submit@mutagens.co.in
researchsubmission@hotmail.com

Research Paper

HISTOPATHOLOGICAL CHANGES IN THE OVARY OF THE BRACKISH WATER FIDDLER CRAB *(Uca annulipes)* EXPOSED TO CADMIUM

V. Suresh[1], A. Palraj[2] and M. Deecaraman[3]

[1]Department of Zoology,
Sir Theagaraya College, Chennai – 600 021
[2]Department of Zoology,
P.G. Department, Govt.Arts and Science College,
Krishnagiri
[3]Department of Industrial Biotechnology,
Dr. MGR University, Chennai- 600 095, India.

Abstract

Cadmium (Cd) is one of the most toxic environmental and industrial pollutants, is known to exert gonodtoxic and spermiotoxic effects. In the present study we investigated toxic effect of cadmium on the ovary of brackish water fiddler crab *Uca annulipes*. The experimental crabs were exposed to sublethal concentrations(1/3rd and 1/10th of 96 hrs LC50) of cadmium (0.013 and 0.04 ppm) for a period of 15 and 30 days, shows many histological changes in ovary. Histological observations of ovary following exposure cadmium showed destruction of epithlial layer, degeneration of oocytes, disorganization of nucleus, vacuolization, shrinkage oocytes rupturing of follicular epithelium and damage of ovarian structure was observal after exposed to sublethal concentrations. Therefore the findings of this investigation can be taken as biomarkers for monitoring heavy metals concentration of aqatic biota.

Key words: Cadmium, Ovary, *Uca annulipes*.

INTRODUCTION

Heavy metal causes severe adverse effects on the health of organisyms. They act at cellular and molecular level which ultimately lead into physiological ant biochemical disorders leading to death. Cadmium (Cd), a widely used heavy metals in modern industry, is one of the most abundant, ubiquitously distributed toxic elements in aquatic system (Novelli *et al*., 2000). The main source of non- occupational exposure to Cd includes smoking and air by which food and water are contaminated by Cd (Nagata *et al*., 2005) World health organization estimates that 4 billion people or 80% of the world population presently use herbal medicine (Naithari *et al*., 2010). In addition, Cd is a common inorganic contaminant of coastal sediments and waters due to anthropogenic pollution and natural sources (Sokolova *et al*., 2004, Ivanina *et al*., 2010). It can be accumulated in aquatic animals (e.g. crab, shrimps, oysters and muscle after entering through different ways such as respiratory tract, digestive tract, surface penetration etc (Daillianis and kaloyianni, 2004, *et al.*, 2008). It is seriously harmful to the growth of aquatic life and survival resulting in decline of their population.

Histological studies have a way for understanding the pathological conditions of the animal by helping in diagnosing the abnormalities or damage of the tissues of the animal exposed to toxic stress of heavy metals (Sprague;1971; Andhale,*et al*.,2011 Maryam,*et al.*,2013). Histological changes not only give an early indication of pollution hazard, but also provide useful data on nature and degree of damage to cues and tissues (Shaikh,2010).

Reproduction is a physiological process and is an essential biological need of animal for the continuity of the generation which is known to dominate all other physiological process. The main function of reproduction is to replace population losses due to death and migration (warren, 1971).

Sarojini *et al*.,(1988) observed the effects of pesticide on different stages in the oogenesis in the freshwater prawn, *Macrobrackhism kristensis*, when expressed to perticide.Yadav and Sarojini (1989) studied the lethal and sublethal effect of endosulfan on the ovary of the freshwater prawn, *Caridina weberi*.

A histological changes in the ovary of the freshwater crab, *Barytelphusa guerini* exposed to cuprous oxide was carried out by Machale *et al.,* 1990. Chourpagar and kulkarni (2011) observed histologicol charges in the tissues of fresh water female crab, *Barytelphusa cunicularis* exposed to heavy metal pesticides.

The available literature reveals that practically less work has been carried out on the effects of cadmium on histopathological aspects of crustacean. Hence, the present study was designed to investigate the histological changes on the ovary of *Uca annulipes* after cadmium exposure.

MATERIALS AND METHODS:

The live specimen of fiddler crab *Uca amnulipes* were collected from pulicat lake of Tamilnadu. They were acclimatized to laboratory conditions under the day night [12L:12D] illumination at 28±2˚C for one week in plastic troughs (20”diameter) containing sufficient brackish water, So that crabs are partially submerged.

Adult and intermoult female crabs (Stage C3; Diwan 1973) having an average carapace length 11.5 ± 0.5 mm and breadth of 15.4± 0.5mm and body weight (25 to 30g) were selected for histopathological studies exposed to LC50 value of cadmium for 24, 48, 72 and 96 hours sublethal concentration (1/3rd and 1/10th LC50 of 96 hr) for 15 and 30 days. For histological study crabs were split into two groups (control, cadmium treated), 10 crabs in each group and maintained under laboratory conditions. The test media was changed daily to maintain cadmium concentration. The animals were fed chronic exposure with boiled beef liver.

Histology:

To study histopathological lesions in the ovary of female crab were dissected immediately and ovary were quickly removed and fixed in aqueous bouin’s fluid. After fixation for 24 hrs. the tissue was passed through alcohol grades for dehydration and cleared in xylene. The tissue was embedded in paraffin wax (M.P 58- 60˚C) and serial sections were cut at 7 to 8µ and stained haematoxylene and eosin.

RESULTS:

Histological structure of control ovary:

The ovary of *Uca annulipes* (Fig.1) is covered with an outer epithelial membrane followed by connective tissue and inner germinative epithelium. In the early stage of development the germinative epithelium. In the early stage of development the germinative zone or zone of proliferation is distinguished by the presence of compact mass of oogonical cells which undergo meiotic division and give rise to primary oocytes(Previtellogenic oocytes). Each vitellogenic oocytes is covered with a thin layer of follicle cells. The mature oocytes or vitellogenic oocytes are completely filled with yolk globules and granules. The nutritive cells are present in close vicinity of the oocytes and supply the nutritive material to the developing oocytes. The degenerating ova are surrounded by nutritive phagocytes which increase in their size with the increase in vacuolization. In fully matured ovary all above described stages of oocytes as well as follicular cells and phagocytes are observed.

Histological changes in the ovary of experimental crab exposed to lower sublethal concentration (0.013 ppm) for 15 and 30 days.

Histological observation of ovary exposure to lower sublethal concentration of cadmium after 15 days showed destruction of epithelial layer and oocytes degeneration and disorganization of nucleus was observed. The cytoplasm exhibited foamy condition was seen (Fig-2A).After 30 days exposure to cadmium, rupturing of oocytes, vacuolization, irregular arrangement of oocytes and disappearance of nucleus was observed (Fig- 2B).

Histopathological changes in the ovary of experimental crab exposed to higher sublethal concentration(0.04 ppm) for 15 and 30 days.

The experimental crabs treated with higher sublethal concentration of cadmium showed many histological changes was observed . After 15 days exposure, pycnosis of nutritive cells and nucleus of oocytes was observed due to shrinkage of oocytes. Destruction of epithelial layer was loosely arranged and vacuolization was also observed in the periphery of the oocytes (Fig-2C). After 30 days exposure ovary showed irregular shape of oocytes, mixing of ooplamic material due to disintegration of follicular epithelium, maximum nature of degenerating oocytes with disintegrated nuclei was observed (Fig -2D)

DISCUSSION

The histopathological changes induced due to cadmium caused specific abnormality at tissue level. In the present study, an attempt was made to study the effect of cadmium caused distinct changes in gonadal histology. The results of the presents study revealed that, ovaries of control crab were covered with outer thin epithelium and inner germinative epithelial layer from which the oocytes proliferate. The oocytes are covered with a layer of follicle cell. The ooplasm is compactly arranged with a thick yolk granules. Ovarian follicles are filled with different types of maturing oocytes (Fig - 1).

In the present study, our investigation demonstrated that cadmium exposure to induced significant alteration in the ovary of *U. annulipes* such as damage of germinal zone, changes in the cell shape, rupture of oocytes membrane, vacuolization, degeneration of tissue, disappearance of nucles and nuclei (Fig – 2A & B). Similar histopathological alterations were reported in different aquatic organism exposed to different pollutants by many investigators. Reddy *et al*., (1982) noticed destruction of epithelial layer, follicle cells, nurse cells, necrosis and degeneration of oocytes in the ovary of fresh water crab *Barytelphusa guerini* when exposed to heavy metals.

After chronic exposure period, lead to increase in damage to the ovarian tissue. The observed cellular deformities following the cadmium exposure may be due to the direct effect on the developing oocytes through general metabolism and growth or through hormone controlling ovarian growth. Similarly, Jadhav and Sheikh (2012) observed exposure and concentrated mediated changes in ovaries of freshwater crab, *Barytelphusa cunicularis* treated with endosulfan. Likewise, Rani *et al.,* (2013) reported degenerative changes in ovaries of mud crab, *Scylla olivacea* when exposed to cadmium nanoparticle. Damage of the ovarian tissue may be due to the direct effects of cadmium with the enzyme system in metabolism or destroying the function of hormone that controlling the ovarian growth and lead to decline reproductive activity.

The observed alteration in the ovarian structure of *U. annulipes* after exposure to cadmium collaborate the earlier reports of several investigators. Shinde et al., (2002), identified the rupturing of oocytes membrane in the oocytes, vacuolization in the peripheral oocytes and distrubances in the supporting connective tissue after acute and chronic exposure of sugar industrial effluent in crab *Barytelphusa gurini*. Deshpande (1985) observed shrinkage in ooplasm, change in cell shape, vacuolization, and degeneration of tissue necrosis of nuclei and increase in number of phagocytes in the freshwater prawn, *M.kistinesis* after exposure to pesticide. Gangshettiwar (1986) observed the effects of phenol on *M.lamerri* and noted rupture of oocytes membrane, changes in cell shape, vacuolization and degeneration of nuclei and nucleus in oocytes.

Histological studies have a way for understanding the pathological conditions of the animal by helping in diagnosing the abnormalities or damage of the tissue exposed to toxic stress of heavy metas (Sprague, 1971; Andhale *et al*.,2011). Histological changes provides an early indication of pollution hazard, and also useful data on nature and degree of damage to cell and tissues (Shaikh *et al*.,2010).

Rao *et al.,* (1987) reported that the inhabition of germinative zone, lack of distinct nucleolus, in oocytes of freshwater prawn, *M.lamerri* after exposure to mercuri chloride. Kharat, *et al*., (2011) observed the histological changes in the ovary of freshwater prawn, *Macrobrachium kistensis* exposed to TBTCL. Histopathological studies are also useful in evaluating the pollution potential of heavy metal pesticides, since trace amount of these chemical which do not bring animal mortality over a given period, were capable of producing considerable organ damage (Kumar and Pant, 1984; Jayakumar, 2002).

Martin *et al*., (1989) observed destruction of proliferating zone and changes in structure of ovary of the freshwater prawn, *C.weberi* exposure to methyl parathion. Yadav and Sarojini (1989) observed that disintegration of follicle epithelium, vacuolization dearrangement of oocytes, pycnosis of nutritive cells of nucleus of oocytes in the ovary of freshwater prawn, *C.weberi* after exposure to endosulfan.

Machale *et al*., (1990) studied the effect of cuprous oxide on the ovary of *B.guerini* and noticed shrinkage in opplasm, vacuolization on peripheral side, rupture of oocytes, structural damage, necrosis of nuclei and nucleoli and disintegration of oocytes. Sarojini *et al*., (1990) observed the degeneration of oocytes, vacuolization and replacement of oogonia with fibror tissue in the overy of freshwater crabs *B.guerini* after exposure to zine sulphate. Similar findings observed on day 30, after lower sublethal concentration of cadmium exposure pycnosis, of nutritive cells and nucleus of oocytes was observed due to shrinkage of oocytes. Destruction of epithelial layer and degeneration of oocytes were seen, epidermal layer was loosely arranged and vacuolization was also observed in the periphery of oocytes (Fig- 2B). On 30 days, higher sublethal concentration the ovary showed irregular shape of oocytes, mixing of ooplasmic materials due to disintegration of follicular epithelium, maximum nature of degenerating oocytes with disintegrated nuclei was observed(Fig- 2B).

In this study histopathological alterations of ovary showed progressive damage in epithelial layer, degeneration of oocytes and its clearly evident with the progress of exposure periods. Damage to ovary, after cadmium exposure leads to developing oocytes interfere with the enzyme system in destroying the function of hormone that controlling the ovarian growth to decline in reproductive activity. Moreover, the findings of present study serve as" biomarkers" for assessing heavy metal toxicity in the aquatic biota.

CONCLUSION

The results of our study indicate that histopathological changes in the ovary showed progressive damage and degeneration and it is clearly evident with the progress of exposure period and extand of tissue damages increase with the increase of cadmium exposure of *Uca annulipes*

ACKOWLEDGEMENT:

Auther are thankful to the principal, Sir Theagaraya College, Chennai- 600021 for providing necessary facilities in the work.

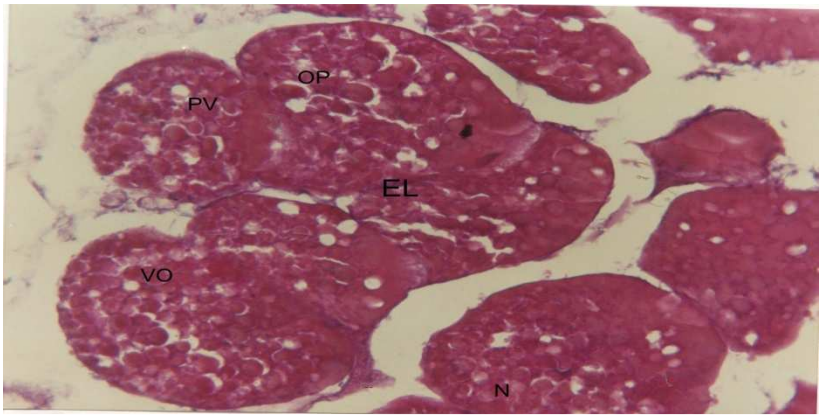

Fig.1 [Control] H & E X100

Fig.1 Photomicrograph of C.S.of control ovary of brackish water carb, *Uca annulipes* showing normal structure of epithelial layer(EL), Vitellogenic oocytes[VO], Nucleus[N], Ooplasm [OP], Previtellogenic oocytes[PV]

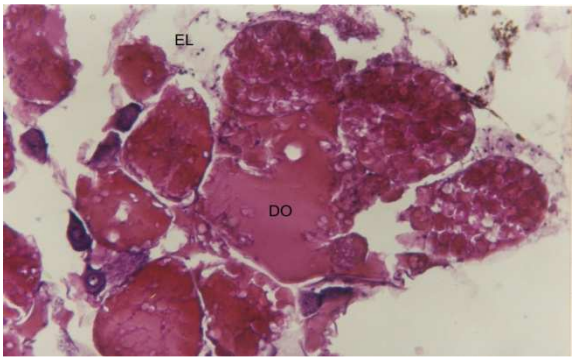

Fig 2 A : L.S.C[15 d] X 100

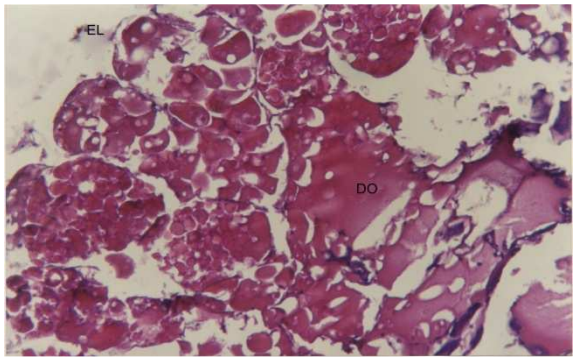

Fig 2 B : L.S.C[30 d] X 100

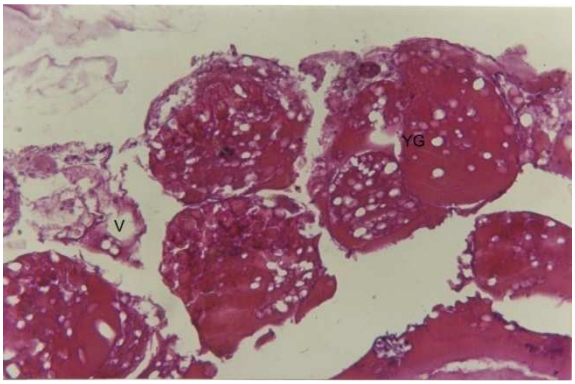

Fig 2 C: H.S.C[15 d] X 100

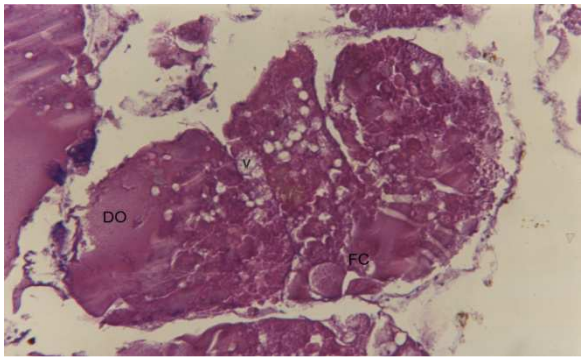

Fig 2 D: H.S.C[30 d] X 100

Fig:2 [A-D] Photomicrograph of C.S. of Ovary in *U annulipes* exposed to lower and higher sublethal concentration of cadmium for showing destruction of Epithelial layer (EL), Degeneration of oocytes [DO], Vacuolization[V], damage of Yolk Granules(YG), follicle cells, necrosis (N) after 15 and 30 days exposure. Stained in haematoxylin and eosin X100.

REFERENCES:

1. Andhale, A.V., Bhosale, P.A. and Zambare, S.P[2011]: Histopathological study of nickel induced alterations in the fresh water bivalve, *Lammellidens merginais.* Journal of Experimental Sciences Vol 2, Issue 4:01-03.
2. Atul R.Chourpagar. [2011]: Toxicity, Bioaccumulation and Detoxification of heavy metal on the freshwater female crab, *Barytelphusa cunicularis* and impact assesment on its

reproduction. Ph.D. Thesis, Dr.Babasaheb Ambedkar Marathwada university, Aurangabad.

3. Dailianis, S. and Kaloyianni, M. [2004]. Cadmium induces both pyruvate kinose and Na+ / H exchanger activity through protein kinase digestive gland cells of Mytilus galloprovincialis (L). J.Experimentl Biol. 207: 1665 – 1674.
4. Deshpande, J.R. [1985]; Effect of some pollutants on the reproduction in crustaceans. Ph.D. Thesis, Dr.Babasaheb Ambedkar Marathwada university, Aurangabad.
5. Diwan, A.D. [1973]; Studies on Sexual maturity and growth rate studies of the freshwater crab, *Barytelphusa cunicularis.* Marath.Univ.J.Sci. 12:125.
6. Gangshettiwar, V.B. [1986]: Effect of Pherol poisoning on the physiology of the prawn, *Macrobrachium lamerrii* Ph.D. Thesis, Dr.Babasaheb Ambedkar Marathwada university, Aurangabad.
7. Ivanina, A.V., Eilers, S. and Kurochkin, I.O. [2010]: Effects of cadmium exposure and intermittent anoxia on nitric oxide metabolism in eastern oysters., Crassostrea Virginica. J. Experimental Biol. 213: 433 – 444.
8. Jadhav T.J and Shaikh, J.D [2012]: Histopathological changes in the ovary of freshwater crab, *Barytelphusa auniaularis* exposed to Endosulphan. Int.J.Sci 1[2] 139-140.
9. Jayakumar, S. [2002]. Effects of copper and zinc toxicity on a fresh water crab *Spiralothelphusa hydrodroma.* Ph.D. Thesis, University of Madras, Tamilnadu, Chennai.
10. Kharat, P.S., Shejule, K.B and Ghobale, B.C. [2011]: Histopathological changes in ovary of freshwater prawn, *Macrobrachium kistensis* exposed to TBTCL. World Journal Zoology, 6(3): 296-300.
11. Kumar, S and Pant, S.C.[1984]: Organal damage caused by adcarb to a freshwater teleost *Barbus conchonius* (Hamilton. Bull. Environ.Contam. Toxicol. 33:50-55.
12. Li, W.Y., Kang, X. and MU,S.M. [2008]. Research advance of toxicological effects of cadmium on shrimps and crabs. Fisheries Sci. 27[1]: 47-50.
13. Machale, P.R., Sarojini, R., Khan, A.K. and Nagabhushanam, R. [1990]: Histological changes in the ovary of freshwater crab, *Barytelphusa guerini* exposed to cuprous oxide. Ind.J. Inv.Zool and Aquat. Biol. 2 (1):55-58.
14. Martin, R.E (1989): Physiological response of the prawn *Caridina weberi* to the pesticides methyl parathion. Ph.D. Thesis, Dr.Babasaheb Ambedkar Marathwada university, Aurangabad, M.S.
15. Maryam, S.M., Khalili, V, Abbasi, A. and Hedayati [2013]: Sublethal effect of copper toxicity on liver lesions of Roach (*Rutilus rutilus caspicus*) Juveniles. JNASCI 2[4] 119-123.
16. Nagata, C., Nagao, Y. and Shibuya, C. [2005]: Urinary Cadmium and Serum Level of Estrogens and Androgens in Postmenopausal Japanere women. Cancer Epidemiol. Biomarkers Prevention. 14 : 705-708.
17. Naithani, V., Pathak, N and chaudhary, M. [2010]: Evaluation of heavy metals in two major Ingrediants of Ampucare. International Journal of Pharamaceutical Sciences and Drug Research. 2(2): 13-141.
18. Novelli, F., Novelli, E., Manzano M.A., Lopes, AM., Cataneo, AC., Barbosa, LL and Ribas, Bo. [2000]: Effect of tocophorol on superoxide redical and toxicity of cadmium exposure . International Journal of Environmental Health Research. 10: 125-134.
19. Rani, D.S, Kavitha, R and Padmaja, M.[2013]: Histological and biochemical changes in reproductive organs of mud crab *Scylla olivacea* [Herbst, 1796] exposed to cadmium nanopartuicle.J.Acad & Indus Resea. Vol.2, (7): 391-396.
20. Rao, S., Sarojini, R., Jayalakshmi, K. and Nagabhushanam [1987]: Effect of Mercuri chloride on the ovarian development in the freshwater prawn, *Macrobrachium lamerri*. Proc.Nat.Symp.Ecotoxical. 123-124.
21. Reddy S.L.N., Shankaraiah, K.V. and Raman Rao: [1982]: Time course alteration in protein metabolism of freshwater field crab, *Barytelphusa guerini*. In proceeding of the all India Symposium on Physiological response of animal of pollutants, Dr.B.A.M.U, Aurangabad.

22. Sarojini, R., Machale, P.R., Khan, A.K and Nagabhushanam, R.[1990]: Effect of cadmium chloride on histology and biochemical content of the hepatopancres of the freshwater crab, *Barytelphusa guerini.* Environmental Science, Vol. IV: 91-97.
23. Sarojini, R., Meena Patil and Nagabhushanam, R. [1988]: Oogenesis and spermatogenesis in the freshwater prawn *Macrobrachium kisten*sis.J.Curr.Biosci, Vol.5(3):83-87.
24. Shaikh, F.I, Ustad, I.R and Ansari, N.T. [2010]: Effect of heavy metal on the ovary of freshwater crab, *Barytelphusa cunicularis* [Westwood]. The Bioscan, 5 [2]: 335- 338
25. Shinde, V.R., Vcersh, M.U., Kulkarni, R.S. [2002]: Ovarian changes in response to heavy metals exposure to the fish *Notopterus notopterus* (pallas). J.Environ. Biol., 23(2), 137-141.
26. Sokolova, I.M., Evans, S. and Hughas, F.M. [2004] Cadmium – induced apoptosis in oyster hermocytes involves disturbance of cellular energy balance but no mitochondrial permeability transition. J. Experimental Biol 207: 3369 – 3380.
27. Sprague, J.B. [1971]: The measurement of pollutant toxicity to fish III, sublethal effect and safe concentration. water Res. 5:245-266.
28. Warren, C.E. [1971]: Biology and water pollution control. W.B.Saunders Co. Philadelphia.
29. Yadav,B.S and Sarojini, R. [1989]: Lethal and Sublethal effect of endosulfan on the ovary of freshwater prawn, *Caridina weberi*. Ind. J. Inv. Zool. Aqua. Biol 1(2) : 98-101.