ENDOCRINE DISRUPTING EFFECTS OF BPA ON REPRODUCTIVE PHYSIOLOGY OF FEMALE FISH HETEROPNEUSTES FOSSILIS

MANISHA SISODIYA, MEENA KHARE, R.R.KANHERE

Abstract: Bisphenol A (BPA) is an important industrial chemical and known as an endocrine disruptor. Earlier reports reveal that it induces both toxic and estrogenic effects in various animal species. Recently, the occurrence of Bisphenol A (BPA) in consumer products and environment has raised concerns about potential adverse effects on reproductive health of both humans and other animal species.

The present study was designed to assess the effects of BPA concentrations consistent with environmental exposure (5, 10, and 20 µg/L) on reproductive physiology in female fish Heteropneustes fossilis. There were no significant differences in body weight among the fishes of various experimental groups. The results of histo pathology of ovary showed that 5 µg/L BPA delayed oocyte maturation. Inhibition of gonadal growth (as measured by the gonadosomatic index) happened in both males and females at concentrations of 10 and 20 µg/L after 21 days. The GSI and ovary weight in fish from the in all experimental groups were significantly lower ($p > 0.05$) than control. Histological observation of the ovary clearly revealed a delay in the onset of oogenesis in fish of experimental animals compared with control. The results clearly exhibit that BPA acts as an endocrine disruptor to fish and adversely affect the reproductive physiology, gonadal development and function of the fish. Furthermore, the reduced GSI was found directly proportional to the BPA. The outcome of this study shows a significant decrease in fecundity and pathological symptoms in all experimental groups than in the control group. These findings are reasonably indicators of reproductive impairments leading to delayed gonadal maturity and negatively affecting processes of ovulation and thus, the fish production.

Keywords: BPA, estrogenic chemical, gonadosomatic index, histology, ovary

I. **Introduction**: Bisphenol A (BPA) is an important industrial chemical and mostly used as an intermediate in the production of polycarbonate (PC) plastics and epoxy resins. It is a chemical and known to be an endocrine disruptor. It has been widely used in the manufacture of polycarbonate plastics, epoxy resins and other industry uses for many years. Due to its major uses in the production of plastic food or beverage containers and the coating of food cans, people of different ages are unavoidably exposed to BPA in daily life. (Schonfelder, G. et al., 2002, Wan et al., 2010, Ikezuki et al., 2002, Yamada et al., 2002, Cao et al., 2012, Sun et al., 2004).

It was reported that, BPA is a xenoestrogen compound that has adverse health effects on the developing reproductive organs, especially if BPA exposure occurred during critical period of development, fetal BPA exposure has been shown to decrease the efficiency of sperm production in male mice offspring (Takahashi and Oishi, 2001 and Chitra et al., 2003). Recent data of Park et al. showed that BPA increased human ovarian cancer cell proliferation in a dose-dependent manner (Park et al., 2009). In the present study, we decided to evaluate the effect of BPA on the reproductive physiology of a female fish. *H.fossilis*.

II. Materials and methods: Chemical reagents: Bisphenol-A (chloroacetone, acetone-d6, acetone, hexafluoroacetylacetone, mercuric iodide red, benzaldehyde, benzimidazole, boron nitride (lab), acetic acid glacial for hplc, 1, 4-dioxan for hplc, acetonitrile 99) were purchased from Alpha Chemika, Mumbai, Maharashtra ,India.

BPA doses: Doses of 5, 10, and 20 µg/L were selected, based on national toxicological report, 1985. BPA is insoluble in water. Therefore acetone was used as a medium to obtain proper distribution in the test solution. A stock solution of BPA was prepared using double distilled water. Serial dilutions of the stock solution were prepared using previously aerated, copper free and stored tap water. The water was continuously aerated. Animals were treated kindly to minimize the laboratory and captive stress.

Animals and treatments: Healthy specimens of freshwater fish *H. fossilis* (Bloch) were procured from local outlets. Fishing was done during late night with the help of trained local fishermen. The collected fish were and transported to the laboratory in ice-cold containers (0–4°C) on the same day. The fish samples were kept alive for at least 24hrs to minimize stress. The specimens had an average weight of 18 ±1.2 g and an average length of 14 ±1.3 cm. They were then treated with 0.05% $KMnO_4$ solution for 2 min to avoid any dermal infection. Test water used in the experiment was analyzed for physico-chemical properties. The fishes were then acclimatized in the laboratory conditions for 15 days and were fed with boiled egg white, goat liver and processed food. The fecal matter and other waste materials were drained off daily to reduce ammonia content in water. They were scarified after 21days of the experiment and ovary was taken out and

processed for histopathological analysis. The stock solution of Bisphenol A was prepared in analytical grade ethanol.

Experimental design: After acclimatization, the fishes were grouped and transferred into four aquaria containing 20L of dechlorinated tap water (n=4). Group I Control (with ethanol), Group II, Group III and Group IV were treated with 5, 10, and 20 µg/L BPA for 21 days.

***Histopathological examination*:** The ovary was fixed in normal saline at room temperature for 24 h before being dehydrated and embedded in paraffin wax (melting point 65°C). The tissues were sectioned at 5, 10, and 20 µg/L and stained with haematoxylin and eosin (H&E). Histopathological abnormalities of ovary were evaluated under a light microscope (NIKON ECLIPSE E 400, USA) and photographed using digital camera attached to the microscope.

Gonadosomatic Index (GSI): It is a calculation of gonad mass as a proportion of total body mass. The Gonadosomatic Index (GSI) was determined (King, 1995) as: GSI = 100 (Gm/Tm) where;

Gm = Mass of Gonad

Tm = Total mass of fish

Mean values were computed and plotted to determine variations. Means were also computed for size groups and test of significant differences. The co-efficient of correlation (r) between fecundity (F) and other variables was tested at the appropriate degree of freedom (d. f.) at 0.001 and 0.05 probability levels of significance.

Results and Discussion: Histopathology is an important tool for measurement of chemical induced alterations in gonads and is considered as an important biomarker in testing of endocrine disrupting chemicals (EDCs). Fish exposed to different sub-lethal concentration of BPA for different exposure periods showed significant level of alteration in the histology of the ovary. These changes were intense and the degree of changes in histology confirmed variation in different concentration of exposure.

Fractional disruption of ovarian follicles and vacuolation in cytoplasm of germinal cells was observed in all experimental groups. The inter follicular connective tissue was damaged. The morphological structure of ovarian follicles got distorted and stretched, losing their typical configuration. Necrosis and fibrosis in connective tissue and damage to yolk vesicles of maturing oocytes was also observed. Degenerative oocytes became phagocytic and exhibited atresia. In maturing oocytes the granulosa layer gets separated and complete or partial rupture was observed. In mature oocytes, clumping of cytoplasm and karyolysis was observed. (Fig.1). The other two sub-lethal exposure results also confirm that, changes in the ovary of *Heteropneustus fossilis* are dose and time dependent. Atresia was seen in the maturing follicles, ovarian follicles separated due to loss of inter-follicular connective tissue. Inter-follicular spaces were larger and vacuolation in developing oocytes were also observed. The degenerating oocytes became phagocytic and formed atretic oocytes. The ovary of group III and IV had shown only few numbers of oocytes and small inter-follicular spaces. Oocytes exhibited vacuolation and atretic condition. Shrinking of cytoplasm, rupture of tunica albugenia, dislocation of nucleus and yolk vesicles was seen. Overall, the results of our study confirm the slow down the proliferation of oocytes and increased in the number of atretic follicles. Necrosis, fibrosis and disintegration in the ovary were high in all experimental fishes especially in higher doses. It is confirmed that BPA affected the secondary growth of primary oocytes due to the destruction of vitellogenesis. Similar histopathological findings were reported in the ovaries of *Anabas testudineus* and C. punctatus after the exposure to sublethal concentration of dimecron (Hossain et al, 2002). Giri et al. (2000) reported the effects of insecticide pesticide induced histopathological anomalies like damage in germinal epithelium, atresia of oocyte, bleeding in stroma and oocytes vacuolization. In 1981, Kling reported that arrest of vitellogenesis, reduction in size of oocytes and resulting in a total atresia of ovaries in *Tilapia leucostica* exposed to labycid. During present study also we found reduction in the numbers of mature oocytes. Our results are similar with the results of Pawar and Katdare (1983) and Muley and Mane (1987) in *Garra mullya* in which sumithion toxicity induced ruptured follicles, completely degenerate nucleus and nucleoli in the gonads of Lamellibranchs under Cythion and malathion toxicity. Rastogi and Kulshrestha(1990) reported necrosis and fibrosis in connective tissue along with dilation of blood vessels in maturing oocytes in carp minnow *Rasbora daniconius* under endosulfan, carbofuran and methyl parathion toxicity. Our results are in agreement with many workers. In *Heteropneustes fossilis* the ovary of control fish was fully matured with large number of mature oocytes. After exposure to BPA the oocytes exhibited degenerative changes, liquification of perinuclear cytoplasm and condensation of nucleus, disappearance of nuclear membrane, cytoplasmic clumping, degenerated granulose layer, and degenerated ovarian wall and wrinkled oocytes. Jyothi and Narayan (1999) observed vacuolation and necrosis, arrested ovarian recrudescence and inter-follicular oedema, in the ovary of *Clarias batrachus* (L) under carbaryl toxicity. Adhesion of primary follicle, cytoplasm shrinkage and clumping, increased atretic oocytes, partial destruction of vitelline

membrane was observed in the ovary of *Heteropneustes fossilis* treated with malathion (Deka and Mahanta, 2012).

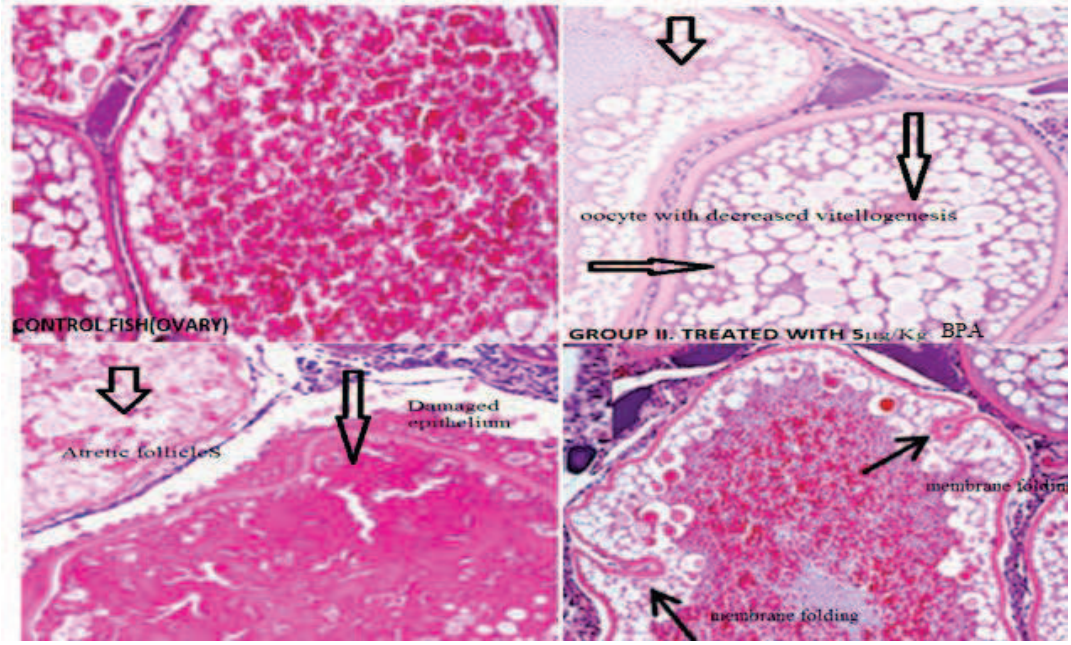

Figure 1. Light photo micrographs of ovaries from H.fossils exposed to various concentrations of BPA showing different histopathological diagnoses. (Haematoxylin-eosin staining.400x)

Gonadosomatic Index: The results of gonadosomatic Index (GSI) in Different Experimental Groups are summarized in Fig.2

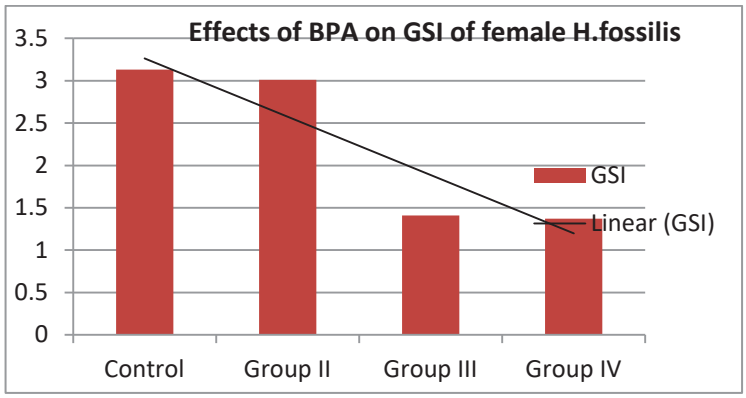

Fig.2: Changes in the mean gonadosomatic indices in control and BPA treated groups of the female *H.fossilis*

***Gonado somatic Index*:** The sequence of maturation events and periodic variation of gonadosomatic ratio (GSI) provides good signal of the level of gonad development with respect to the time of year. Various stages of gonad on an explanatory scale permit a quick qualitative assessment of the breeding condition and gonad weight gives a quantitative record of changes in the gonad state (Crossland, 1977). Our results clearly shown that a decrease in GSI values was found in all experimental groups as compared to control groups (Fig, 2) Results reveal a slight initial increase in the values of GSI but in experimental groups the GSI value was significantly decreased. ($P<0.01$). It may be also prominent that GSI did not much deviate significantly from the control value until after 10days of BPA exposure. However, 21 days of the experiment GSI values differed significantly from the control values.

***Conclusion*:** The current study demonstrated that BPA caused reproductive toxicity which is clearly evident in histopathology of ovary. Our data revealed that BPA caused striking impact by decreasing the GSI compared to their activities in the control group. In consistent with the previous data, we observed histopathological changes in the ovary indicating variable damage due to exposure to BPA. Our microscopic examination also revealed that the ovary could be susceptible to even low doses. A decrease in

GSI, smaller and less developed oocytes, fewer mature oocytes, increase in number of atretic follicles, break down of yolk granules and broken zona radiata was also observed in all experimental fishes especially fish treated with higher doses. In a conclusion, low levels of BPA by time will have an inhibitory effect on the reproduction, decreasing the fecundity of fish. Therefore from the obtained results, we concluded that exposure to environmental relevant concentrations of BPA resulted in a significant damage of ovary in *H.fossilis*. Although, the selected doses of BPA were relevant to their concentration in environment, we feel that their levels, even at very low concentrations, may cause harmful effects on human health too.

References:

1. Cao, X.L., J. Zhang, C.G. Goodyer, S. Hayward, G.M. Cooke. (2012). Bisphenol A in human placental and fetal liver tissues collected from Greater Montreal area (Quebec) during (1998–2008), Chemosphere, 89, pp. 505–511.
2. Chitra, K.C., K.R. Rao, P.P. Mathur. (2003). Effect of Bisphenol A and co-administration of Bisphenol A and vitamin C on epididymis of adult rats: a histological and biochemical study, Asian J. Androl., 5, pp. 203–208.
3. Crossland, J. (1977). "Fecundity of the Snapper Chrysophrys auratus (Pisces; Sparidae) from the Hauraki Gulf". N.Z.J. Mar. Freshwater Res., 11(4):67-775.
4. Deka, S. and Mohanta.R. (2012). A study on the Effect of Organophosphorous Pesticide Malathion on Hepato-Renal and Reproductive Organs of Heteropneustes fossilis (Bloch). The science Probe. 1(1): pp.1-13.
5. Giri A.N., Srivastava D.K. and Trivedi S.P., 2000. Insecticide basathrin induced histoanatomical insult of ovarian tissue of Indian catfish, Heteropneustes fossilis. Biol. Memoirs., 26, 20-24.
6. H. Yamada, I. Furuta, E.H. Kato, S. Kataoka, Y. Usuki. (2002). Maternal serum and amniotic fluid Bisphenol A concentrations in the early second trimester. Reprod. Toxicol. 16, pp. 735–739.
7. Hossain, z., Rehman, M.Z.and Mollah.M.F.A. (2002). Effect of Dimecron100 SCW on Anabas testudineus, Channa punctates and Barbodes gonionotus.Ind.J.Fish.49, 415-417.
8. Jyothi, B. and Narayan, G. 1999. Toxic effects of carbaryl on gonads of freshwater fish, Clarias batrachus (Linnaeus). J. Environ. Biol. 20(1): 73-76.
9. Muley, D. V. and Mane, U. H. (1987). Histopathological changes induced by cythion-malathion in the gonads of Lamellibranch mollusks. Environ. Ecology. 5(4): 756-759.
10. Park, S.H. K.Y. Kim, B.S. An, J.H. Choi, E.B. Jeung. (2009).Cell growth of ovarian cancer cells is stimulated by xenoestrogens through an estrogen-dependent pathway, but their stimulation of cell growth appears not to be involved in the activation of the mitogen-activated protein kinases ERK-1 and p38, J. Reprod. Dev., 55 (2009), pp. 23–29.
11. Pawar, K. R. and Katdare, M. (1983). Effect of sumithion on ovaries of fresh water fish, Garra mullya (Sykes). Current Science. 56, (12): 784- 785.
12. Rastogi, A. and Kulshrestha, S. K. (1990). Effect of Sublethal Doses of Three Pesticides on the Ovary of a Carp Minnow Rasbora daniconius Bull. Environ. Contam. Toxicol. 45: 742-747.
13. Schonfelder, G, G., W. Wittfoht, H. Hopp, C.E. Talsness, M. Paul. (2002). Parent Bisphenol A accumulation in the human maternal-fetal-placental unit. Environ. Health Perspect. 110, pp. A703–A770.
14. Sun, Y. M. Irie, N. Kishikawa, M. Wada, N. Kuroda. (2004).Determination of Bisphenol A in human breast milk by HPLC with column-switching and fluorescence detection. Biomed. Chromatogr, 18 (2004), pp. 501–507.
15. Takahashi, O and Oishi, S. (2001). Testicular toxicity of dietary 2, 2-bis (4-hydroxyphenyl) propane (Bisphenol A) in F344 rats. Arch. Toxicol., 75, pp. 42–51.
16. Wan, Y. K. Choi, S. Kim, K. Ji, H. Chang, S. Wiseman, P.D. Jones, J.S. Khim, S. Park, J. Park, M.H. Lam, J.P. Giesy. (2010). Hydroxylated polybrominated diphenyl ethers and Bisphenol A in pregnant women and their matching fetuses: placental transfer and potential risks. Environ. Sci. Technol., 44, pp. 5233–5239.

Manisha Sisodiya,
Department of Zoology, Govt. PG College, Jhabua (M.P), India
Meena Khare
Department of Zoology, Mata Jeeja Bai Govt. P.G. Girls College, Moti Tabela, Indore, (M.P),
R.R. Kanhere
Govt. Girls College, Barwani (M.P), India.