TFR
Trends in Fisheries Research
An International Peer-Reviewed Journal
UGC Approved Journal No: 63495
www.sciencejournal.in

HEPATOTOXIC EFFECTS OF BISPHENOL A ON H.FOSSILIS

Manisha Sisodiya[1], Meena Khare[2] and R.R.Kanhere[3]

[1] Department of Zoology, Govt. PG College, Jhabua (M.P), India
[2]Department of Zoology, Mata Jeeja Bai Govt. P.G. Girls College, Moti Tabela, Indore, (M.P),
[3]R.R. Kanhere, Vice Chancellor, MP *Bhoj University* (M.P), India.
(E-mail: drmaanaa420@gmail.com)

ABSTRACT
Bisphenol A (BPA) is one of major industrial chemical and known endocrine disruptor. The present study was intended to assess the biochemical and haematological effects of BPA in a catfish *H.fossilis.* Healthy fishes of 36.78g in weight were exposed to various concentrations of Bisphenol A to calculate the medium lethal concentration LC_{50} value using probit analysis method and it is found as 7.1443 mg/l. Fish was exposed to different sublethal concentrations i.e. to 0.714 mg/L (1/10), 1.428 mg/L (1/20) and 2.142 mg/L (1/30%) (Group II, III and IV) using ten specimens in each aquarium for 45 days. After termination of the experiment fish were dissected and liver was taken out for histopathological and biochemical observations. Blood sample was collected by severing the caudal peduncle and processed for various primary and secondary blood indices. Total plasma protein content was also estimated.

The results confirmed that BPA was moderately toxic to the fish *Heteropneustes fossilis* and toxic effects are found to be depend on both time and concentration dependent. The treatment of sub lethal concentrations of BPA induced significant changes in the histopathology of liver. After an initial increase in all experimental groups, the RBC, WBC and Hct contents fallen a significant decrease in all exposed groups. Exposure to graded concentrations of BPA showed reduction in plasma protein levels in response to various BPA treatments. The reduction in haematological parameters may be due interference of BPA in the synthesis of haemoglobin or might have caused erythrolysis or it may be binding with to alter the secondary structure of haemoglobin. The decrease in RBC count may be due to destruction of synthesized RBC or due to impaired synthesis of RBC or may be due to the toxic effects of BPA on cell membranes. The present study confirms that sub lethal concentrations of BPA induced various histopathological, haematological and metabolic anomalies in *H.fossilis*. In conclusion, we recommend the use of biochemical and histopathological studies as bioindicators of EDC contamination for better understanding of EDC exposure.

KEYWORDS: Bisphenol A, haematology, histopathology, endocrine disruptor.

INTRODUCTION

India has 16 per cent of the world's population and four per cent of its fresh water resources. Several fresh water sources in India are facing rapid eutrophication due to filling of surplus nutrients, sedimentation, acidification and the introduction of industrial and municipal toxicants (Reddy, P.B and Baghel, B.S, 2012, Kumar, D. and Thakur, M.K., 2017). Overall, water pollution effects living organisms that live in these water bodies and the harmful effects are not only to the individual species but also to the natural biological communities. It happens when contaminants are discharged directly or indirectly into water bodies without adequate treatment (Reddy, P.B. and Rawat, S.S., 2013). The entry of these pollutants into aquatic systems involves a major threat to water quality and biological characteristics of the aquatic ecosystems.

Bisphenol A (BPA) is one of the major industrial chemical and known endocrine disruptor. Owing to its major uses in the production of plastic and food and beverage containers people are indisputably exposed to BPA in daily life. Research on bisphenol A (BPA) as an endocrine disruptor and ecological pollutant has now major regulatory implications toward the ecosystem health (Canesi, L. and Fabbri, E., 2015). Like several other industrial chemicals, fresh water basins are the major sinks for bisphenol A (BPA) (Reddy, P.B. and Baghel, B.S., 2012, Reddy, P.B., 2012a, b, Reddy, P.B., 2013, Srivastava, B. and Reddy, P.B. 2017). According to a report of U.S. Environmental Protection Agency (USEPA), one million pounds of BPA are released into the atmosphere per annum and found in municipal wastewater in low quantities (Erler C, Novak J (2010).

Trends in Fisheries Research
An International Peer-Reviewed Journal
UGC Approved Journal No: 63495
www.sciencejournal.in

The results of on-going laboratory research also raised the concern about the probable threats of BPA (Bisphenol A) as an endocrine disruptor as it closely mimics the structure and function of the hormone estradiol by binding to and activating the same estrogen receptor as the natural hormone(Takayanagi *et al*, 2006,Takahashi *et al*, 2018). Therefore, the present study was designed to determine haematological, biochemical and histopathological changes in liver of a catfish, *H. fossilis* to assess probable effects of Bisphenol A.

MATERIALS AND METHODS

Healthy adult fish were picked up during spawning season of 2016 from local commercial aqua culture ponds and were acclimatized to the laboratory conditions for 15 days.Bisphenol-A*2,2-Bis (4-hydroxyphenyl) propane (CAS* Number: 80-05-7)97% pure was procured from Shreeji Pharma International, Vadodara, Gujarat, India. Groups of 10 healthy fishes (mean wt. 36.78g) were exposed to different concentrations of Bisphenol A to calculate the medium lethal concentration LC_{50} value using probit analysis method (Finney, 1952). The LC_{50} of BPA for *H.fossilis* in this experiment was found to be 7.142 mg/L. The fish was exposed to different sublethal concentrations of BPA (0.714 mg/L (1/10), 1.428 mg/L (1/20 and 2.142 mg/L (1/30%) i.e. (Group II, III and IV) for 45 days using ten specimens in each aquarium. After the termination of experiment, fish was dissected and liver was taken out for histopathological examination. Blood samples were collected from all experimental fishes through caudal vein puncture by using Ethylene diamine tetra acetic acid (EDTA) as an anticoagulant.

Hematological Analysis:

An aliquot of the blood was utilized for the estimation of various haematological parameters like erythrocyte count (RBC), haematocrit (PCV), haemoglobin (Hb), and Leucocyte count (WBC). The secondary indices like mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) was calculated from the average values of Hb %(Dacie and Lewis, 1984). RBC count was made by using a Neubauer crystalline counting chamber as described by Blaxhall (1972).Haemoglobin concentration was measured by the cyanmethaemoglobin method using a commercially available kit (Span, India).

Haematocrit (Hct %) (Packed red blood cell volume) (PCV) was assessed immediately by Wintrobe method (Wintrobe, 1974).International Normalized Ratio (INR) was measured based on blood clotting time. The total plasma protein content was assessed according to modified standard method by (Lowery *et al*., 1951).

Histopathology:

Fixed liver tissue was processed for in graded ethanol series, cleared in xylene and finally embedded in paraffin wax (melting point 60^{o}C). The embedded block were sectioned at 6µ on a rotary microtome, mounted on glass slides, dried and stained with haematoxylin and eosin (H&E). Sections were examined under a light microscope (NIKON ECLIPSE E 400, USA) and photographed by using digital camera attached to the microscope.

RESULTS

Determination of Lethal Concentration $(LC)_{50}$ of Bisphenol A for *Heteropneustes fossilis*:

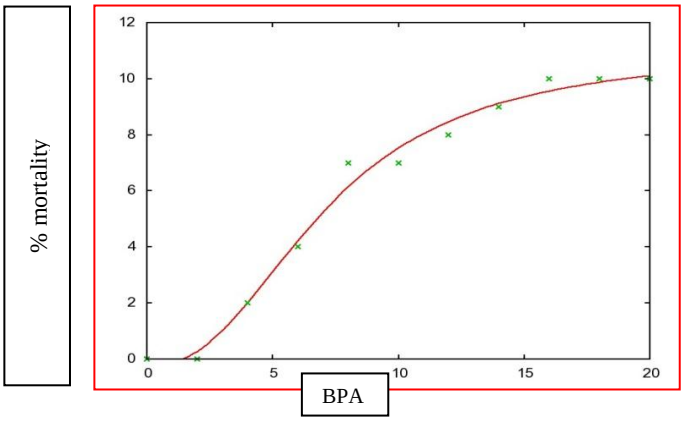
Results according to probit analysis showed the median lethal concentration LC_{50} of estimated Bisphenol A to *Heteropneustes fossilis* for 96 h of exposure to be 7. 114 mg/l, the lower bound and upper bound 95% lethal confidence limits for BPA indicated a wide range of values (5 to 10 mg/L).

Effect of BPA on hematological parameters:

The results of hematological values of both control and experimental groups in *H.fossilis* (means ± SEM) for RBC's and WBC's count, haemoglobin (Hb) values and haematocrit (Hct) rates, mean erythrocyte volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) are shown in Table 2. After an initial increase in Group II, the values of all blood indices like RBC, WBC and Hct contents significantly decreased in exposed groups. Exposure to graded concentrations of BPA showed significant reduction in plasma protein levels also.

The INR (International Normalized Ratio, for reporting the results of blood coagulation (clotting) values are also followed similar trend in all experimental groups.

Trends in Fisheries Research
An International Peer-Reviewed Journal
UGC Approved Journal No: 63495
www.sciencejournal.in

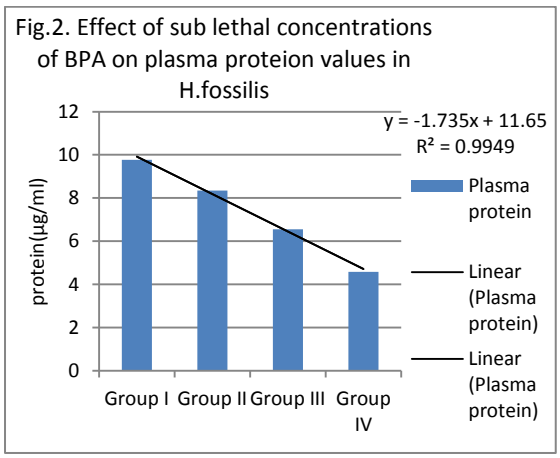


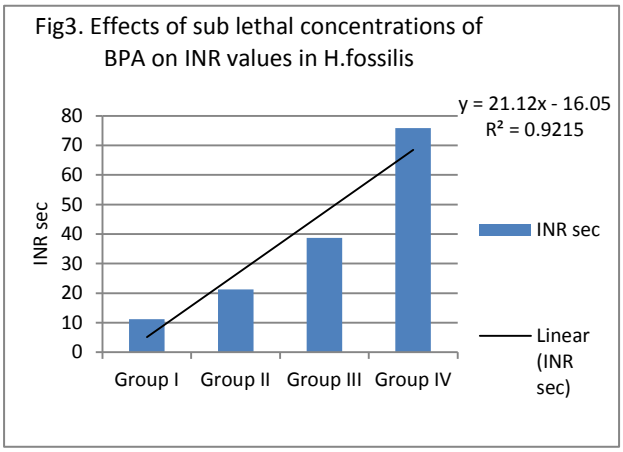
Minimum	-0.225589 +/- 0.4154 (184.2%)
Maximum	10.9621 +/- 0.781 (7.125%)
LC_{50}	7.1443 +/- 0.5814 (8.138%)
Hill coeff.	2.40789 +/- 0.4516 (18.75%)

Figure 1. Determination of LC50 value of Bisphenol A for *Heteropneustes fossilis* (http://www.ic50.tk/).

Table.2. Changes in the hematological parameters in *H. fossilis* treated with sub lethal concentration of bisphenol A.

Parameter	Control (Group I)	Group II	Group III	Group III
RBC (million/cu mm)	1.72 ± 0.058	1.9 ± 0.104	1.44 ± 0.024	1.58 ± 0.086
WBC (1000/cu mm)	25.17 ± 0.748	43.02 ± 0.299	42.06 ± 0.624	49.59 ± 0.327
Haemoglobin (g/dl)	4.595 ± 0.100	3.321 ± 0.309	2.692 ± 0.071	2.21 ± 0.041
Hematocrit (%)	17.46 ± 0.722	13.14 ± 0.201	10.84 ± 0.515	9.52 ± 0.193
MCV(fL) femtoliters	106.81±3.1	95.14±1.4	83.61±1.7	77.27±1.4
MCH (pg/cell)	32.55±0.75	27.15±0.48	26.36±0.61	22.33±0.96
MCHC (g/dL)	38.86±0.65	39.48±1.14	31.12±0.82	25.16±0.76
Plasma protein(µg/ml)	9.77±0.95	8.34±0.24	6.56±0.63	4.58±0.61
INR (sec)	11.2 ± 0.33	21.3 ± 0.51	38.7 ± 1.09	75.8 ± 2.1





TFR
Trends in Fisheries Research
An International Peer-Reviewed Journal
UGC Approved Journal No: 63495
www.sciencejournal.in

Histopathological studies: The results of histopathological examinations are summarized in Fig.2. Fish liver from control group showed normal architecture. However, the liver exhibited several histopathological changes in a dose-dependent manner in exposure to various sublethal concentrations of BPA. Fish exposed to 0.714 mg/L (Group II) sublethal concentration of BPA illustrated vacuolation, necrosis, degeneration, ruptured central vein and few broken hepatocytes. Aggregation of melanomacrophages (MMC) and higher amount of glycogen granules was also seen. Liver of fish exposed to 1.428 mg/L of BPA (Group III) exhibited nuclear vacuolation, cytoplasmic degeneration, cellular rupture, congestion of blood vessels, nuclear degeneration, pyknotic nucleus, bile stagnation. Glycogen granules are less in amount. Massive number of macrophage penetration was also seen. As the dose increased, decrease in glycogen granules was observed with complete absence in the fish group IV exposed to of 2.142 mg/L of BPA. In Group IV, the changes were extreme with vacuolated and pyknotic nucleus. Nuclear and cytoplasmic degeneration and melanomacrophages aggregates were also found. The histopathological signs resulted in the sub-lethal treatment of BPA evidently implies that the BPA is capable to modify the liver histology in fish.

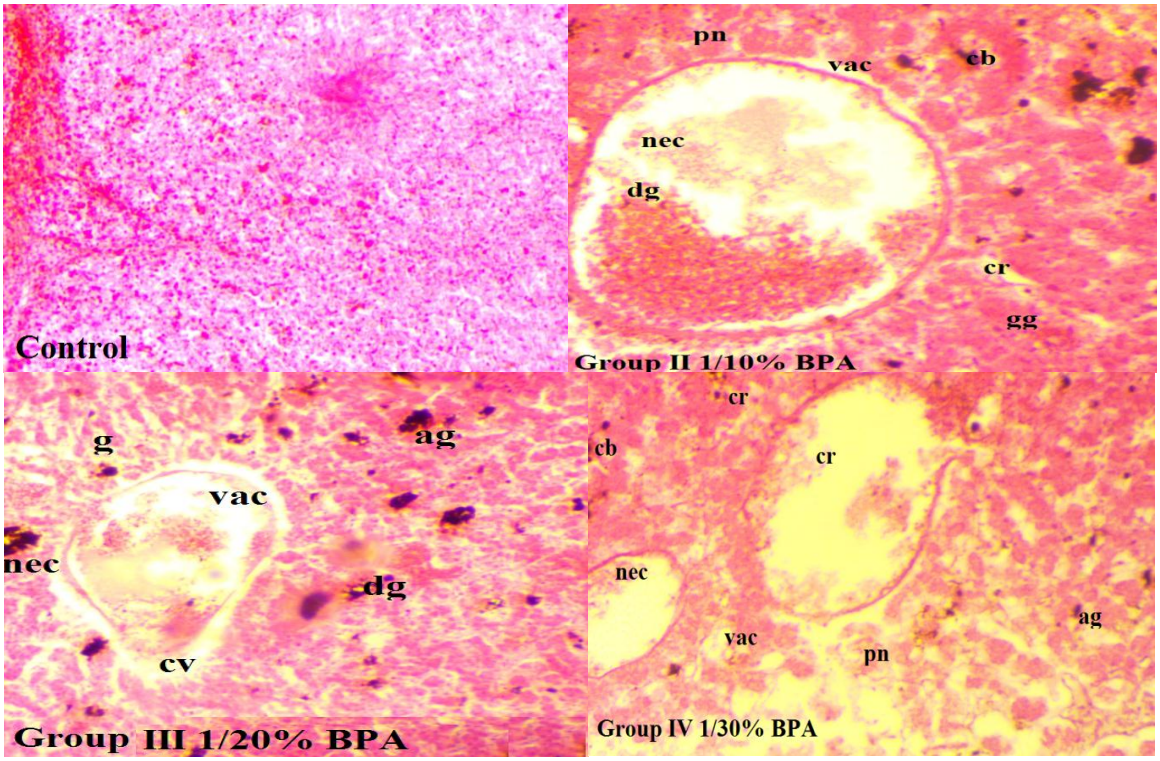


FIG. 2. Effects of sub lethal concentration of BPA on histopathology of liver of *Heteropneustes fossilis*. A. Control fish show normal structure. B. Liver from Group II showing swelling. (x 1000) C. Histology of liver of *Heteropneustes fossilis* from Group III. Showing acute cellular swelling, hypertrophy and pyknosis .D. Showing acute cellular swelling, hypertrophy and pyknosis).Secondary infections in between hepatocytes are also seen. (All sections are H&E Stained x400).

DISCUSSION

Bisphenol A contamination in the aquatic environment may be due to untreated wastewater from industrial units (Kang *et al.,* 2007). In general, the LC_{50} values of Bisphenol A range between 3-15mg/L for fresh water teleosts (Caspers, 1998). The LC_{50} values of Bisphenol A (99%) for the fish *Heteropneustes fossilis* for 24, 48, 72 and 96h in static exposure are 18.1 mg/L, 15.11mg/L, 14.80 mg/L and 7.114 mg/L respectively. However it is 6.48mg/L in Cichlid fish, *Etroplus maculatus (*Asifa and Chitra 2015), 6.8 and 8.3 mg L in adult male and female Japanese medaka, Oryzias latipes (Kashiwada *et al*, 2002), 6.669 mg/L in zebrafish, Danio rerio (Mai, 2016) and 6.323 mg/L a freshwater fish,

Trends in Fisheries Research
An International Peer-Reviewed Journal
UGC Approved Journal No: 63495
www.sciencejournal.in

*Ctenopharyngodon idella (*Faheem and Lone, 2017).The variation in LC_{50} value of the same substance against same or different species, or the LC_{50} value of different chemicals against same or different fish species is maybe due to the design, formulation and stereochemistry of the toxicant or their active molecules (https://ehs.unl.edu/). The intensity of toxicity also depends on water quality parameters, health, size, weight, age and dietetic status of the fish (Reddy 2012[a], 2016[b]).

Earlier, studies have confirmed the severe effects of various anthropogenic toxicants including BPA on various organs of fish (Faheem *et al.,* 2016; Reddy, 2017; Ratn *et al,* 2018).The information of hematological features is an important tool and an effective sensitive index for the assessment of physiological and pathological changes in fishes (Reddy, 2012, Reddy, 2013). The results of the present study, revealed that there was a significant ($P < 0.05$) reduction in hematological (haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin) and biochemical (protein) parameters in the BPA treated fish compared to control fish. The reduction in haematological parameters may be due interference of BPA in the synthesis of haemoglobin or might have caused erythrolysis or it may be binding with to alter the secondary structure of haemoglobin, which sequentially leads to the decrease in the oxygen carrying capacity of the blood. The reduction in the haemoglobin content will finally leads to oxygen stress causing numerous lethal effects to the organism (Aiswarya *et al,* 2016). The decrease in the RBCs number may be due to decrease in haemoglobin content of blood. The decrease in RBC count may be due to destruction of synthesized RBC or due to impaired synthesis of RBC or may be due to the effects of BPA on cell membranes, which caused injury or apoptosis to the blood cells. Our results are in agreement with many workers (Reddy, 2013, Mekkawy *et al,* 2011, Aiswarya *et al,* 2016).The erythrocyte deformations might cause low oxygen level and caused respiratory dysfunction or stress which might alter the erythrocyte number and morphology as in African catfish (*Clarias gariepinus*) treated with sub lethal concentrations 4-nonylphenol (Mekkawy *et al,* 2011). Nevertheless, to understand the changes in the values of blood parameters is quite difficult as they are affected by both internal and external factors (Bastami *et al,* 2009, Xu *et al,* 2013, Huggett, R.J., 2018).The reduction in RBC, Hb and PCV subsequently led to reduction in oxygen carrying capacity of blood and causing to anaemic condition in the fish. Our result confirms the findings of Adhika**ri *et al,*** (2004), Witeska (2015), Bhatnagar *et al,* (2017) and Osman *et al,* (2018) who confirmed the anaemic status, may be because of inhibition of erythropoiesis, damaged erythrocytes and haemosynthesis in haemopoietic organs. Furthermore, reduction in RBC, Hb and PCV may be endorsed to haemolysis/haemodilution, which is a mechanism of diluting the haem concentration to reduce the effects of toxicants (Shahi, J., et al, 2013, Narra, M.R., et al, 2017, Olchowik-Grabarek, *et al,* 2018).

Haemoglobin (Hb), mean corpuscular haemoglobin (MCH) and mean corpuscular (MCV) levels were significantly increased initially in Group I fish compared to control fish, which is probably due to the initial oxidative stress of BPA in the exposed fish. However, the level of these parameters was lowered in Group III and IV because of the adaptation of fish. Overall, BPA exposed fish showed, a higher WBC count compared to control fish. This means that BPA can stimulate some non-specific immune factors in fish resulting in a better condition for the fish to resist potential pathogens. In the present investigation, the total WBC count is noticeably increased in BPA treated (experimental) fish, which may be due to initiate the defence mechanism of the fish to combat the stress induced by toxicant. The higher values of WBC may be to enhance wound healing, inflammatory disease, parasitic infections and viral diseases. Due to limitations of the adaptive immune system, poikilothermic nature, inadequate stock of antibodies and the slow maturation, proliferation and memory of their lymphocytes the innate immune response in fish is desperately wanted to combat pathogens (Whyte, 2007**:** Uribe *et al,* 2011).An inverse relationship between WBC and RBC counts was observed in the control and experimental groups. Nevertheless, the differences in various blood parameters may be endorsed to several biotic (such as age, season, maturity, pathogens) and abiotic (including water temperature, pH, dissolved oxygen content) and in particular to stress (Sharma *et al,* 2017**;** Zhelev *et al,* 2018).

In the present investigation, it was evident that there were delays in the blood coagulation times, as well as decreases in elasticity of the clots formed, after the exposure of the fish (*H. fossilis*) to sub lethal concentrations of BPA. The findings of this study confirm that BPA is very toxic to *H. fossilis* and caused liver damage. The liver is also responsible for the production of coagulation factors. The International Normalized Ratio (INR) measures the speed of a particular pathway of coagulation, comparing to normal. In the present investigation, the INR values were high and increased gradually in dose dependent manner in all experimental fishes. It clearly indicates that due to the damage of liver in fish of polluted zones the synthesis of vitamin K- dependent coagulation factors have been impaired hence clotting time was delayed. Reddy and Baghel (2012), observed similar results in *Mystus tengara* exposed to industrial

effluents. Nussey *et al,* (1995) in Oreochromis mossambicus (Cichlidae) after exposure to sub lethal concentrations of copper sulphate.

Histopathological investigations have been evidenced as sensitive tool to identify direct toxic effects of chemical compounds within target organs of fish both in laboratory and field investigations (Reddy and Singh, 2011, Reddy, 2012, Reddy and Baghel, 2012, Reddy and Kusum, 2013, Reddy and Rawat, 2013).Liver is the key player and linked with the detoxification and biotransformation process (Reddy, 2012, 2013, Ratn *et al*, 2018).The fish, *H. fossilis* exposed to different sub lethal concentrations of BPA exhibited changes in the normal architecture of liver, dilated blood vessels and sinusoids, congestion of central vein, increased vacuolization and necrosis. Change in normal liver architecture with dilated sinusoids may be due to loss of structural proteins. Abnormal liver structural changes were observed in the fish Siganus *rivulates* exposed to heavy metals (Abdelaziz et al. 2006). Vacuolation of hepatocytes in the present study may be a nonspecific response of fish due to BPA toxicity (Faheem *et al*, 2016). The vacuolization of hepatocytes might indicate unevenness between the speed of synthesis of substances in the parenchyma cells and the rate of their release into the circulation (Faheem, M., et al, 2016, Kanwal *et al*, 2018). Cellular degeneration and necrosis in hepatocytes in the present study may be due to the congestion of central vein. Results of the present study clearly revealed that chronic exposure to BPA induces abnormal development and disrupted metabolism as in other teleosts (Canesi and Fabbri, 2015; Sadoul *et al*, 2017; Pandey *et al*, 2018).

CONCLUSION

H. fossilis is a hardy fish and capable of booming against various antagonistic environmental conditions. Still, the present research confirms that sub lethal concentrations of BPA induced various histopathological, haematological and metabolic abnormalities in *H.fossilis*. The improvement and standardization of biochemical and histopathological assays can be employed as bioindicators of EDC contamination for better understanding of EDC exposure. Further research at molecular level with multiple biomarkers is necessary to support the data. The present research could be very much helpful to treat affected fish to improve health and quality of fishes and other organisms in polluted aquatic system.

REFERENCES

Abdel-aziz S.H., N. El-ghazaly and G.A. Bindohaish (2006). Effect of pollutants in coastal water of Jeddah on the histological structure of liver of the fish Siganus rivulatus. *Saudi Arabia. Egypt. J. Aquat. Res*. 32: 316-333.

Adhikari S., Sarkar B., Chatterjee A., Mahapatra C.T. and Ayyappan S., (2004). Effects of cypermethrin and carbofuran on certain hematological parameters and prediction of their recovery in a freshwater teleost, Labeo rohita (Hamilton). *Ecotoxicol. Environ. Safety*. 58(2):220-226.

Aiswarya K.S. and James R. (2016). Effect of Bisphenol A on certain Hematological Parameters of Heteropneustes fossilis, Bloch. *Int. J. Emerging Trends Sci. Tech.* 3 (8):4493-4497.

Bastami, K.D., Moradlou A.H., Zaragabadi A.M., Mir S.S. and Shakiba M.M. (2009). Measurement of some haematological characteristics of the wild carp. *Comparative Clin. Pathol.* 18(3):321-323.

Bhatnagar A., Cheema, N. and Yadav, A.S., (2017). Alterations in Haematological and Biochemical Profile of Freshwater Fish, Cirrhinus mrigala (Hamilton) Exposed to Sub-lethal Concentrations of Chlorpyrifos. *Nature Environ. Pollut. Tech.* 16(4):1189-1194.

Blaxhall P.C. (1972).The haematological assessment of the health of freshwater fish (J). *J. Fish Biol.* 4: 593-600.

Canesi L. and Fabbri E., (2015). Environmental effects of BPA: focus on aquatic species. *Dose-Response.* 13(3): p.1559325815598304.

Caspers N. (1998). No Estrogenic Effects of Bisphenol A in Daphnia magna STRAUS. *Bull. Environ. Contamination Toxicol.* 61:143-148. Testing laboratory: Bayer AG, Leverkusen, Germany.

Dacie J.V and Lewis S.M. (1984). Practical haematology, 6th edn. ELBS and Churchill, Livingston, 1984.

Erler C and Novak J. (2010). Bisphenol A exposure: human risk and health policy. *J Pediatr Nurs* 25(5):400–407.

Faheem M., Khaliq S. and Lone, K.P., (2016). Non-monotonic endocrine disrupting effects of bisphenol-A on vitellogenin expression in juvenile freshwater cyprinid, Catla catla. *Pakistan J. Zoo.* 49(4):1531-1534.

Finney D. J. (1952). Probit Analysis. Cambridge, England, Cambridge University Press.
https://ehs.unl.edu/

Huggett R.J., (2018). Biomarkers: biochemical, physiological, and histological markers of anthropogenic stress. CRC Press

Kang J.H., Aasi D. and Katayama Y. (2007). Bisphenol A in the aquatic environment and its endocrine-disruptive effects on aquatic organisms. *Critical Riv. Toxicol.* 37(7): 607-625.

Kumar D. and Thakur M.K. (2017). Effect of perinatal exposure to Bisphenol-A on DNA methylation and histone acetylation in cerebral cortex and hippocampus of postnatal male mice. *The J. Toxicol. Sci.* 42(3):281-289.

Lowry O.H, Rosebrough N.J, Farr A.L. Randall R.I. (1951). Protein measurement with folin phenol reagent. *J. Biol. Chem*. 193: 265-275.

Mekkawy I.A., Mahmoud U.M. and Sayed A.E.D.H. (2011). Effects of 4-nonylphenol on blood cells of the African catfish Clarias gariepinus (Burchell, 1822). *Tissue Cell.* 43(4):223-229.

Narra M.R., Rajender K., Reddy R.R., Murty U.S. and Begum G. (2017). Insecticides induced stress response and recuperation in fish: Biomarkers in blood and tissues related to oxidative damage. *Chemosphere.* 168:350-357.

Nussey G., Van Vuren J.H.J. and Du Preez H.H. (1995). Effect of copper on blood coagulation of Oreochromis mossambicus (Cichlidae). Comparative Biochemistry and Physiology Part C: Pharmacology. *Toxicol. Endocrinol.* 111(3):359-367.

Olchowik-Grabarek E., Makarova K., Mavlyanov S., Abdullajanova N. and Zamaraeva M., (2018). Comparative analysis of BPA and HQ toxic impacts on human erythrocytes, protective effect mechanism of tannins (*Rhus typhina*). *Environ. Sci. Pollut. Res*. 25(2):1200-1209.

Osman A.G., AbouelFadl K.Y., Abd El Baset M., Mahmoud U.M., Kloas W. and Moustafa M.A. (2018). Blood Biomarkers in Nile tilapia Oreochromis niloticus and African Catfish Clarias gariepinus to Evaluate Water Quality of the River Nile. *J. Fisheries Sci.* 12(1).

Pandey M., Ghorai, S.M. and Rai U. (2018). Bisphenol A mediated effects on innate immunity in freshwater teleost spotted snakehead Channa punctatus murrel. *Fisheries Sci.* 84(1):25-31.

Ratn A., Prasad R., Awasthi Y., Kumar M., Misra A. and Trivedi S.P. (2018). Zn 2+ induced molecular responses associated with oxidative stress, DNA damage and histopathological lesions in liver and kidney of the fish, Channa punctatus (Bloch, 1793). *Ecotoxicol. Environ. Safety*. 151:10-20.

Reddy P.B. (2013). Assessment of Water Quality Using Haematological indices as Biomarkers. *Int. J. Pharmaceut. Biol. Arch.* 4 (2).

Reddy P.B. (2016). Assessment of seasonal and anthropogenic impact on the surface water quality and health status of fish in Chambal River at Nagda, MP India. *Shodhasarita,* Vol.1, No.III pp88-97.

Reddy P.B. (2016). Study of pollution induced oxidative stress in a Cat Fish (*Mystus tengara)*. *European J. Biomed. Pharmaceut. Sci.* 3(12): 595-600.

Reddy P.B. and Baghel B.S. (2012). Impact of Industrial wastewater on the Chambal River and Biomarker responses in fish due to pollution at Nagda. MP India. *DAV Int. J. Sci.* 1(1):86-91.

Reddy P.B. and Kusum W. (2013). Using histopathology of fish as a protocol in the assessment of aquatic pollution. *Journal of Environmental Research and Development*, *8*(2), p.371.

Reddy P.B. and Rawat S.S. (2013). Assessment of aquatic pollution using histopathology in fish as a protocol. *Int. Res. J. Environ. Sci.* 2(8): pp.79-82.

Reddy P.B. and Singh, R.K.,(2011). Biomarker responses in fish exposed to industrial effluent. In "Green Technology and Environmental Conservation (GTEC 2011), 2011 *Int. Con.* (pp. 191-204). *IEEE.*

Reddy P.B. (2012) a. Evaluation of potential biomarkers for effluent induced hepatotoxicity. *Int. J. Applied Bioeng. 6*(2).

Reddy P.B., (2012) b. Histopathogical studies as potential and direct biomarkers of pollution. *Trends Life Sci.* 1(1):27-31.

Reddy P.B., Ishaque S. and Subhashini V. (2012). Plasma circulating nucleic acids (CNAs) of fish as potential biomarkers of toxicity. *Trends Biotech.Res.*1(2): 12-15.

Reddy P.B. (2017). Statistical approach for the assessment of water quality parameters of Chambal River at Nagda. *Life Sciences Int. Res. J.* 4 Spl Issue. 38-41.

Sadoul B., Birceanu O., Aluru N., Thomas J.K. and Vijayan M.M. (2017). Bisphenol A in eggs causes development-specific liver molecular reprogramming in two generations of rainbow trout. *Scientific Reports 7*(1):14131.

Shahi J., Chauhan S. and Singh A., (2013). Comparative study on the haematological effect of synthetic and plant origin pesticides on fish Channa punctatus. *Ind. Jrnl. Nat. Prod. Resources.* 4 (1): 48-53.

Sharma J., Dar S.A., Sayani, A.N. and Langer S., (2017). Effect of Stressors on Haematological and Hormonal Parameters of Garra gotyla gotyla. *Int. J. Curr. Microbiol.App. Sci*. 6(5): 357-369.

Srivastava B. and Reddy P.B. (2017). Lipid peroxidation and DNA damage as biomarkers of pollution induced oxidative stress (OS) in fish. *Life Sci. Int. Res. J.* 4(1): 194-198.

Takahashi M., Komada M., Miyazawa K., Goto S. and Ikeda Y., (2018). Bisphenol A exposure induces increased microglia and microglial related factors in the murine embryonic dorsal telencephalon and hypothalamus. Toxicology letters, 284, pp.113-119.

Uribe C., Folch H., Enriquez R. and Moran G., (2011). Innate and adaptive immunity in teleost fish: a review. *Vete. Medicina*, 56(10):486-503.

Whyte S.K. (2007). The innate immune response of finfish–a review of current knowledge. *Fish & shellfish Immunol.* 23(6):1127-1151.

Wintrobe M.M. (1974). Clinical haematology. Lea and Febiger, Philadelphia, 1974.

Witeska M. (2015). Anemia in teleost fishes. *Bull. Eur. Ass. Fish Pathol*. 35(4), p.149.

Xu X., Ding X., Zhang X., Su S., Treiber F.A., Vlietinck R., Fagard R., Derom C., Gielen M., Loos R.J. and Snieder H. (2013). Genetic and environmental influences on blood pressure variability: a study in twins. *J. Hypertension.* 31(4).

Zhelev Z. Tsonev, S., Georgieva K. and Arnaudova D. (2018). Health status of Pelophylax ridibundus (Amphibia: Ranidae) in a rice paddy ecosystem in Southern Bulgaria and its importance in assessing environmental state: haematological parameters. Environmental Science and Pollution Res. 1-12.

