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DELETERIOUS EFFECT OF SHORT TERM EXPOSURE TO XENOESTROGEN- BISPHENOL A ON CERTAIN HAEMATOLOGICAL AND PHYSIOLOGICAL PROFILE OF FRESHWATER MURREL, *CHANNA STRIATA* (BLOCH, 1793)

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ABSTRACT

Used worldwide intermediately for the production of polycarbonate plastics and epoxy resins, Bisphenol A (BPA) has found its way into the aquatic ecosystems via leaching. With studies already confirming the endocrine-disrupting capability of BPA, the present investigation was focused on the effects of different sublethal concentrations (0.1 ppm, 0.2ppm and 0.4 ppm) of BPA on the haematological, and physiological parameters of freshwater murrel, *Channa striata* on the 7th and 21st day of its exposure. To assess the impact of BPA, it's lethal concentration (LC50) was determined first and was found to be 4.13ppm. The study here reports the capability of BPA to depress the haematological parameters of the fish studied. BPA exposure also elicited a significant decrease in the level of protein as well as cholesterol in the muscle and liver. Dose-dependent degenerative changes were also observed in the architecture of BPA exposed fish liver. In conclusion, BPA induced stress conditions were found to adversely affect the functioning of *Channa striata*.

KEY WORDS: *Channa striata,* Bisphenol A, Red blood cell, White blood cell, Protein, Liver.

INTRODUCTION

Since the rediscovery of Bisphenol A (BPA) in the early 1950s for the development of polycarbonate plastics (Schnell, 1959), its production has been increasing tremendously and is estimated to reach approximately 10.6 million metric tones by 2022 (Research and Markets, 2016). BPA, a xenoestrogen, has become ubiquitous in the environment despite its short half-life (2.5 to 4 days) due to its continuous release (Oehlmann *et al.,* 2009). Used worldwide in the production of a multitude of products like paper coatings, food and beverages packaging, electronic components, flame retardants, building materials, and adhesives, BPA has found its way into various components of the environment via leaching from these products under normal conditions of use (Markey *et al.,* 2002).

With aquatic ecosystems being the ultimate sinks of most anthropogenic chemicals, BPA has been detected in different waters at varying concentrations such as in the effluents of wastewater treatment plants (0.097- 370.0 µg L^{-1}), drinking water (882.5 µg L^{-1}), soil leachates (128.0-200.5 µg L^{-1}), landfill leachates(less than 17.2 µg L^{-1}) as well as surface waters (nondetect to 56 µg L^{-1}) (Corrales *et al.,* 2015; Li *et al.,* 2017; Given that fishes occupy a major part of the aquatic ecosystem, they are considered the primary risk organisms most likely exposed to BPA which may then pose serious threats to their nutritive quality as well as their physiological functions.

Most of the studies conducted in fish related to BPA toxicity have focused on the endocrine-disrupting and estrogen-mimicking capability of BPA (Caballero-Gallardo *et al.,* 2016; Qiu *et al.,* 2016; Wang *et al.,* 2019; Weber *et al.,* 2015). With respect to such studies, very few data have been published on

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the changes in the haematological, histological and physiological profile of fishes exposed to BPA (Faheem *et al.,* 2016; George *et al.,* 2017b; Makinwa and Uadia, 2017). Moreover, the bioaccumulation of BPA is found to be species-specific (Lee *et al.,* 2015). The striped snakehead, *Channa striata,* being one of the most prized freshwater food fish species is found widely distributed in different parts of southern Asia, southern China, Indochina, and the Sunda Islands (Courtenay Jr. and Williams, 2004). However, very little information is available regarding the toxicity of BPA on *Channa striata* and hence the present study is conducted to evaluate the effect of short term exposure of BPA on the haematological, and physiological profile of *Channa striata*. These investigations may be used as indicators to determine the health of the species, status of water quality and potential risks they might face.

MATERIALS AND METHOD

Experimental Model: The adult fish *Channa striata* (Order: *Perciformes,* Family: *Channidae*) procured from a farm at Changanassery was initially treated for 3 mins with 0.05% $KMnO_4$ solution to get rid of any dermal infections. They were then transferred to concrete tanks filled with dechlorinated freshwater for 1 week and fed with minced goat liver so as to get them acclimatized to the laboratory conditions. The experimental models were maintained under standard conditions (26 ± 3 ^{0}C, pH 7 ± 0.37, 12 hr light/dark cycle) on a recirculating water system. The water was replenished every 24 hours to remove leftover food particles. Dead fish were promptly removed. Healthy fish irrespective of sex but of comparable body length (20 ± 2 cm) and weight (80 ± 4 g) were selected for the study.

Test substance: Bisphenol A ($C_{15}H_{16}O_2$) (Molar mass: 228.29 gm/mole) for the toxicity study was produced from the Central Drug House, Mumbai (Product No. 028643, Purity 97%).

Estimation of median lethal concentration (LC_{50}): Acute toxicity tests were conducted in order to calculate the 96 hr LC_{50} for BPA, using probit analysis (Finney, 1971). Group of 12 fish was exposed to different concentrations of BPA for 96 hrs during which the test medium was not renewed. The test concentrations were prepared by serial dilution and it included those concentrations which killed all the fish within 24 hrs and those which did not kill any of the fish within 96 hrs. The probit value of percentage mortality on the y-axis was plotted against the logarithm of test chemical concentration on the x-axis and the LC_{50} was determined by fitting a regression equation as well as graphical interpolation using the Microsoft Excel Software (Fig. 1). The LC_{50} value of BPA for Channa striata was found to be 4.13 ppm.

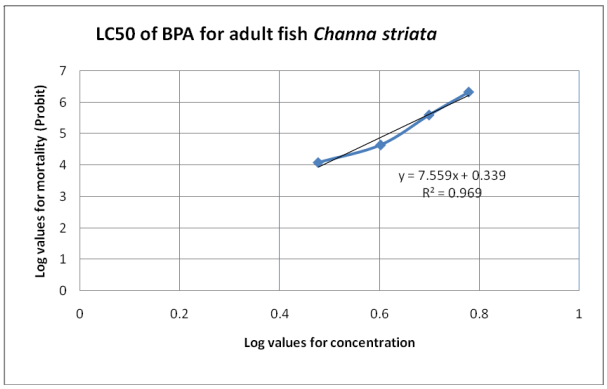

Fig. 1. Log concentration for 50% mortality was found via probit analysis to be 0.616

Experimental Protocol for the exposure study: Experiments were conducted in 20 L Aquaria fiberglass tanks containing 10 L chlorine-free water with different sub-lethal concentrations of BPA (i.e., 0.1 ppm, 0.2 ppm and 0.4 ppm) and control (no BPA). Laboratory acclimatized healthy fishes were introduced into these gently aerated semi-static systems. Each of the short-term exposures (7 and 21 days) were conducted in triplicate for the control as well as three of BPA concentrations. Each tank housed 6 fish (n=6) and feeding was allowed throughout the experimental period. Every 24 hr, half of the water was siphoned and settled fish waste was removed. This was replaced by clean water with the same BPA concentrations.

Blood sample collection and haematological assay: By the end of the experimental period (7 and 21 days), fish were killed by decapitation and the blood was collected in Eppendorf tubes containing EDTA anticoagulant. Fresh unclotted blood was used to find the number of erythrocytes (RBC) and leukocytes (WBC) by the Neubauer hemocytometer using Shaw's solution (Hesser, 1960). Hemoglobin (Hb) content was determined by Sahli's haemoglobinometer based on acid haematin method. Blood samples taken from the caudal fin without anticoagulant was quickly smeared onto a glass slide and stained using Giemsa stain for

counting of lymphocytes. Lymphocyte numbers were then determined by direct counting under the microscope with the aid of the Neubauer chamber. Mean cell volume (MCV), Packed cell volume (PCV) and Mean cell hemoglobin (MCH), the derived haematological indices, were estimated using standard formulae (Jain, 1993).

Biochemical assay: Muscle tissues and liver were removed and blotted free of blood for the estimation of protein and cholesterol. Folin – Ciocalteu method (Lowry *et al.*, 1951) was used for the quantification of protein using photoelectric calorimeter. The estimation of cholesterol was made using spectrophotometer based on the modified Yoshimatsu reaction method (Negrin, 1969).

Histopathological analysis: Liver of fish were dissected out and preserved in Bouin's fixative. The preserved tissues were dehydrated by treating them in various grades of alcohol followed by clearing them with xylene. The samples were then embedded in paraffin wax and using a microtome, sections were cut at 5mm thickness. The sections were double-stained with haemotoxylene and eosine. The stained slides are examined with the help of a compound microscope. Photomicrographs were taken using a camera fitted to a binocular compound microscope (Leica DM750).

Statistical analysis: All data pooled through this study were expressed as means ± standard deviation (X ± SD). A minimum of six replicates was taken for each parameter. For all parameters analyzed, differences between means of the control and BPA exposed groups were determined using Student's *t*-test. All statements of significance were defined at $p<0.05$.

RESULTS

Effect of Bisphenol A on serum haematological variables: From the study, the main haematological response of *Channa striata* to different sublethal levels of BPA exposure (0.1 ppm, 0.2 ppm and 0.4 ppm) was a reduction in RBC count, WBC count and lymphocyte count compared to the control groups. BPA exposed fish had a significant decrease in its haemoglobin content also. The derived haematological indices, packed cell volume, mean corpuscular volume and mean corpuscular haemoglobin content, also depicted reduction in BPA exposed groups with respect to the control group. Significant reduction in the haematological parameters was reported in the 0.2 and 0.4 ppm BPA exposed fish (Table 1).

Effect of Bisphenol A on biochemical variables: BPA has been observed to significantly lower the protein levels in the muscle and liver of exposed fish in a dose-dependent manner when compared to the control fish group. Evaluation of the muscle and liver cholesterol levels has also been found to significantly decrease in the BPA exposed groups of fish (Table 2).

Effect of Bisphenol A on histochemistry of liver: Fig 2. A depicts a photomicrograph showing the histology of the control group fish liver. The control liver exhibited a normal architecture and there were no pathological abnormalities. The hepatocytes

Table 1: Hematological parameters ($\bar{x}$ ± SD) in control and BPA exposed fish.

Parameter	Days	Control	0.1ppm	0.2ppm	0.4ppm
RBC Count (millions/mm^3)	Day 7	3.633±0.07	3.433±0.13	2.733±0.34	*1.156±0.09
	Day 21	3.633±0.07	2.733±0.07	2.083±0.06	*1.234±0.08
WBC Count (cells/mm^3)	Day 7	14203.33±41.44	10169±18.240	9268.5±36.98	*8731.5±75.82
	Day 21	14203.33±41.44	9388.83±67.95	*7518.83±165.58	*3300.16±204.11
Hemoglobin (g%)	Day 7	13.75±0.09	12.26±0.31	*8.31±0.05	*7.7±0.04
	Day 21	13.75±0.09	11.26±0.09	*6.15±0.22	*4±0.17
Lymphocyte (%)	Day 7	97.83±0.52	97.33±0.36	95.83±0.43	93±0.4
	Day 21	97.83±0.52	96.16±0.52	94±0.4	*90.5±0.46
Packed cell volume (%)	Day 7	37.5±0.83	35.83±0.33	34.83±1.24	32.33±0.73
	Day 21	37.5±0.83	27.33±0.92	*26±0.4	*21.33±0.46
Mean cell volume (fL)	Day 7	117±0.77	116.16±0.59	109.33±0.67	*93.83±0.52
	Day 21	117±0.77	107.66±0.92	*96±0.63	*91.66±0.61
Mean cell hemoglobin (pg)	Day 7	36.66±0.832	35.83±0.95	32±0.8	*29.33±0.36
	Day 21	36.66±0.832	36.66±0.61	31.83±0.65	*28.5±0.836

*significant ($p<0.05$)

Table 2. Biochemical parameters (± SD) in control and BPA exposed fish

Parameters	Days	Control	0.1ppm	0.2ppm	0.4ppm
Muscle protein (mg/g)	Day 7	18.05±0.44	17.31±0.18	15.48±.34	*13.18±0.51
	Day 21	18.05±0.44	16.60±0.05	14.53±0.07	*12.43±0.05
Liver protein (mg/g)	Day 7	14.65±0.06	13.31±0.05	10.51±0.08	*8.42±0.03
	Day 21	14.65±0.06	12.57±0.04	*8.59±0.06	*6.343±0.05
Muscle cholesterol (mg/100g)	Day 7	90.79±3.39	83.43±4.22	*74.97±2.15	*63.3±2.15
	Day 21	90.79±3.39	80.74±2.39	*69.13±2.41	*61.99±1.70
Liver cholesterol (mg/100g)	Day 7	202.55±8.56	193.19±6.60	*172.04±2.82	*160.66±3.52
	Day 21	202.55±8.56	192.79±4.02	*168.86±2.43	*154.77±2.07

*significant ($p<0.05$)

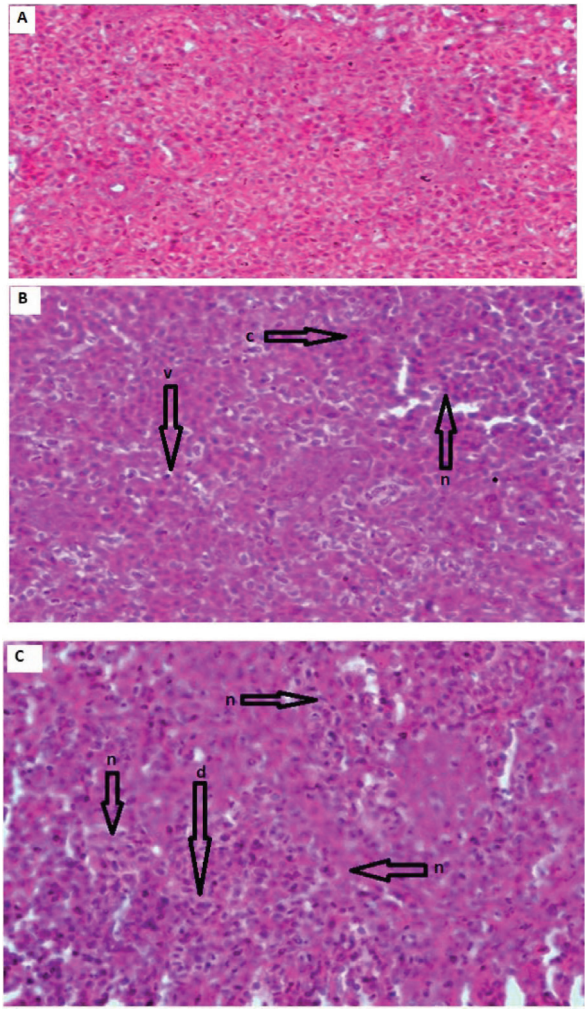

Fig. 2. Histological changes in the liver of experimental fish *Channa striata.* (A) Section of control liver depicting normal trabecular hepatocytes (X 40 magnification) (B) liver histology of 21 days bisphenol A (0.2 ppm) treated fish showing slight necrosis (n), congestion (c), and vacuolization (v) in hepatocytes (X 40 magnification) (C) Liver histology of 21 days bisphenol A (0.4 ppm) treated fish showing disorganization (d) and widespread necrosis (n) in hepatocytes (X 40 magnification)

present had a homogeneous cytoplasm and large central or sub-central nucleus. Only fish exposed for 21 days to BPA (0.2 ppm and 0.4 ppm) showed variations from the normal histology of fish liver.Treatment with 0.2 ppm BPA for 21 days resulted in slightly congested architecture as well as localized cytoplasmic vacuolization and necrosis of hepatocytes (Fig. 2 B). 21 days exposure to 0.4 ppm BPA showed widespread necrosis of hepatocytes. Disorganization and congestion of hepatocytes were also observed at this sublethal concentration (Fig. 2 C).

DISCUSSION

Blood being the medium of intercellular and intracellular transport, which comes in direct contact with various organs and tissues of the body, is capable of reflecting the physiological state of an animal at a particular time. Red blood cells (RBCs) in addition to being transporters of oxygen and nutrients from lungs to tissues are important interorgan communication systems (Kuhn *et al.*, 2017). In order to find out the effect of BPA on the ability of RBC to carry out these functions, the RBC count, haemoglobin concentration and mean corpuscular haemoglobin (MCH) concentration have been measured in BPA exposed and the control fish group. The study found BPA exposure to cause a decrease in the RBCs of all BPA exposed fish groups with a significant reduction in the highest sub-lethal concentration (0.4ppm) studied. The results were found to be consistent with data from the study of Keum *et al.*, (2005). This decrease in the RBC might be probably due to the capacity of BPA to induce oxidative damage to bone marrow cells which are responsible for the production of erythrocytes (Tiwari and Vanage, 2017) or might be due to the promotion of haemolysis. Studies have demonstrated the capacity of BPA and its analogs, in

humans, to promote eryptosis (RBC programmed death) by increasing the translocation of phosphatidylserine into RBCs along with an increase in the activity of calpain and caspase-3 (Macczak *et al.,* 2016). BPA being an endocrine disruptor, another possible mechanism is the impairment of erythropoiesis via inhibition of the secretion and signaling of glycoprotein hormone erythropoietin (Pal *et al.,* 2017). The results in our study suggests that BPA exposure probably induced anemic hypoxia and carbon dioxide toxicity in the tissues of exposed fish.

Fish haemoglobin found in erythrocytes, has the same molecular architecture as that of mammalian haemoglobin, with a heme content and under most conditions tetrameric (Giardina *et al.,* 1973). In our study, BPA exposure resulted in a marked reduction in haemoglobin concentration. The reduction in erythrocytes, as found in this study, is the obvious reason for the reduction in haemoglobin concentration. BPA probably induced haemolysis of RBC, releasing Hb out to the plasma, where it gets catabolised to bilirubin and gets finally excreted out (Hastuti *et al.,* 2019). Our results, of BPA induced reduction in Hb concentration, is in line with those observed in other studies (Kaliappan *et al.,* 2017; Keum *et al.,* 2005). The significant decrease in derived haematological indices, PCV, MCV, and MCH, imply further the possible shrinkage of RBCs as a result of BPA induced toxic stress.

WBCs are the main warriors of the fish immune systems that help fight infections and also defend the body in the presence of foreign materials. WBC count would help assess the impact of BPA on the immune system. Typically as seen in other studies (Kaliappan *et al.,* 2017; Rogers and Mirza, 2013), in the presence of a toxic substance (such as BPA), an increase in the concentration of WBCs as a result of activation or stimulation of the immune system would be expected. However, our study reports contradictory results, showing a significant reduction in the WBCs of BPA exposed fish which is indicative of a suppressed immune system. Studies have demonstrated the immunotoxicity of BPA exposure with results indicating reduction in the number of macrophages and lymphocytes due to the exposure (Sugita-Konishi *et al.,* 2003). Invitro, BPA has been shown to hinder the mitogenesis of lymphocytes (Sakazaki *et al.,* 2002) and the production of monocyte chemoattractant protein- 1 (MCP-1) (Inadera *et al.,* 2000). In our study also, BPA exposure resulted in a slight decrease in lymphocyte counts. Even at lower doses BPA was found to affect the immune system by disrupting the immune system cell and cytokine levels (Özaydin *et al.,* 2018). This is possibly due to the xenoestrogen activity of BPA, whereby it binds to estrogen receptors α (ER-α) and β (ER-β) (Bauer *et al.,* 2012) which are found on immune system cells including dendritic cells, macrophages, and lymphocytes (Nalbandian and Kovats, 2005). The potential of BPA to alter macrophage adhesion thereby inhibiting the functioning of macrophage has been demonstrated in-vitro (Segura *et al.,* 1999).

Proteins serve several functions including providing architectural support via protein mass, as enzymes which help catalyze metabolic reactions, as signaling molecules within and between cells and also as fuel to support survival. Protein synthesis and degradation are closely regulated and gets affected by physiological and pathophysiological conditions (Liu and Barrett, 2002). To examine the effect of BPA on the protein involved physiological functions, the protein content of the muscle and liver tissues were examined in all BPA exposed and control groups. Significant reduction in protein content was observed in the highest sublethal dose exposed (0.4 ppm). Depletion of protein content due to toxicant stress has been reported in several studies (Ahmed *et al.,* 2015; George *et al.,* 2017a, 2017b). BPA was found to induce endoplasmic reticulum (ER) stress-associated apoptosis, thereby interrupting protein synthesis (ER is an important organelle for protein synthesis) (Asahi *et al.,* 2010). Another plausible explanation for the marked reduction in protein content is the downregulation of translation elongation factors vital for protein synthesis as a result of BPA induced oxidative stress (Chienwichai *et al.,* 2018). The mechanistic target of rapamycin (mTOR) signaling pathway is an important pathway in the protein synthesis machinery. Several studies have reported BPA to interfere with mTor regulation in different cell types (Boucher *et al.,* 2014; Goodson *et al.,* 2011; Ribeiro-Varandas *et al.,* 2014), in turn affecting the protein synthesis.

Balanced cholesterol levels are important in maintaining good health as they are necessary for the production of hormones, vitamin D, bile as well as maintaining the structure of cell membranes. In order to study the impact of BPA exposure on the metabolic functions of the fish, the cholesterol levels in the liver and muscle tissues were measured. Significant reduction in the cholesterol level was

observed during the exposure period. This might probably be due to the impairment of cholesterol biosynthesis in the liver or due to the reduced absorption of dietary cholesterol. Several studies have reported a decrease in cholesterol levels upon exposure to toxic substances (Binukumari and Vasanthi, 2014; Shakoori *et al.,* (1996) states that this decrease might be due to the use of fatty deposits instead of glucose for energy purposes. A similar effect of decreased cholesterol was observed in Korean rockfish, *Sebastes schlegeli* when subjected to 10 mg BPA per body weight (Keum *et al.,* 2005).

The liver is one of the most common targets for cytotoxicity and tumorigenesis, mainly due to biotransformation reactions occurring in it which enhances toxicity of metabolites (Faheem *et al.,* 2016; Hinton *et al.,* 2017). Histopathological investigation of the teleost liver would reflect the overall health status of the fish studied and is found to be an accurate way to assess the impact of any pollutant on the fish. BPA exposure in fish is found to result in the generation of oxygen free radicals in its liver, thereby inducing lipid peroxidation and modifying the antioxidant status consequently exerting toxic effects in a time-dependent manner (Chitra and Maiby, 2014). In the present study the control liver, as expected, exhibited a normal architecture and there were no pathological abnormalities. The section of control liver depicted normal trabecular hepatocytes, while that of 21 days BPA exposed (0.2 and 0.4 ppm) groups showed hepatocellular necrosis, congestion, and cytoplasmic vacuolization as well as disorganization of hepatocytes. Changes in the normal architecture of the fish liver may be attributed to the loss of structural protein upon toxicant exposure (Faheem and Parvez Lone, 2017). Vacuolation of hepatocytes, which indicates an imbalance between the rate of synthesis of substances in the parenchyma cells and the rate of their release into the circulation (Gingerich, 1982), is found to be a non-specific response of fish after exposure to a toxicant (Roberts, 2012). Accumulation of lipids and glycogen in hepatocytes as a result of aquatic pollution might also cause widespread vacuolization. Similar deposition of glycogen in the hepatocytes is commonly found in stressed fishes, as glycogen acts as a reserve of glucose to supply the high energy demand occurring during such situations (Hinton and Lauren, 1990). The excessive detoxification done by the fish liver to get rid of the BPA from its body might be the possible cause for necrosis of hepatocytes noticed in BPA exposed fish liver. The inability of the exposed fish to regenerate new liver cells might be another possible reason for the widespread necrosis. Similar degenerative changes to hepatocytes were observed in *Catla catla* (Faheem *et al.,* 2019), *Heteropneustes fossilis* (Sisodiya *et al.,* 2018) and *Oreochromis mossambicus* (Deepa *et al.,* 2017) upon BPA exposure. The histopathological changes noted in the present study is in accordance with observations made in fishes during exposure to different endocrine disruptors as well as other toxicants (Abdel-Warith *et al.,* 2011; Faheem and Parvez Lone, 2017; George *et al.,* 2017a; Kumar and Nandan, 2014).

Considering the results obtained from the present study, it can be concluded that BPA exposure impaired the haematological as well as physiological functions of the freshwater murrel, *Channa striata.*

REFERENCES

Abdel-Warith, A. A., Younis, E. M., Al-Asgah, N. A. and Wahbi, O. M. 2011. Effect of zinc toxicity on liver histology of Nile tilapia, *Oreochromis niloticus. Sci. Res. Essays*. 6(17): 3760-3769.

Ahmed, W. M. S., Moselhy, W. A. and Nabil, T. M. 2015. Bisphenol A Toxicity in Adult Male Rats: Hematological, Biochemical and Histopathological Approach. *Glob. Vet.* 14(2): 228-238.

Asahi, J., Kamo, H., Baba, R., Doi, Y., Yamashita, A., Murakami, D., Hanada, A. and Hirano, T. 2010. Bisphenol A induces endoplasmic reticulum stress-associated apoptosis in mouse non-parenchymal hepatocytes. *Life Sci.* 87(13-14): 431-438.

Bauer, S. M., Roy, A., Emo, J., Chapman, T. J., Georas, S. N. and Lawrence, B. P. 2012. The Effects of Maternal Exposure to Bisphenol A on Allergic Lung Inflammation into Adulthood. *Toxicol. Sci.* 130(1): 82-93.

Binukumari, S. and Vasanthi, J. 2014. Changes in cholesterol content of the freshwater fish, *Labeo rohita* due to the effect of an insecticide "Encounter" (Herbal plant extract). *Int. J. Pharm. Sci. Res.* 5(2): 397-399.

Boucher, J. G., Husain, M., Rowan-Carroll, A., Williams, A., Yauk, C. L. and Atlas, E. 2014 . Identification of mechanisms of action of bisphenol a-induced human preadipocyte differentiation by transcriptional profiling. *Obesity.* 22(11): 2333-2343.

Caballero-Gallardo, K., Olivero-Verbel, J. and Freeman, J. L. 2016. Toxicogenomics to Evaluate Endocrine Disrupting Effects of Environmental Chemicals Using the Zebrafish Model. *Curr. Genomics*. 17(6): 515-527.

Chienwichai, P., Topanurak, S., Reamtong, O., Boonyuen, U., Worakhunpiset, S. and Tharnpoophasiam, P. 2018. Effects of low bisphenol A concentration on protein expression profiles in an in vitro model of non-alcoholic fatty liver disease. *Mol. Cell. Toxicol.* 14(1): 61–72.

Chitra, K. C. and Maiby, S. 2014. Oxidative stress of Bisphenol-A and its adverse effects on the liver of fresh water fish *Oreochromis mossambicus*. *Int. J. Sci. Res.* 3(7): 221-224.

Corrales, J., Kristofco, L. A., Steele, W. B., Yates, B. S., Breed, C. S., Williams, E. S. and Brooks, B. W. 2015. Global Assessment of Bisphenol A in the Environment: Review and Analysis of Its Occurrence and Bioaccumulation. *Dose-Response. An Int. J.* 13(3): 1-29.

Courtenay Jr., W. R. and Williams, J. D. 2004. Snakeheads (Pisces, Channidae)- A Biological Synopsis and Risk Assessment. U.S. Geological Survey Circular 1251, Denver, Colorado. pp 2.

Deepa, G., Evans, D. A. and Sunny, F. 2017. Bisphenol A Induced Alterations in the Liver and Protein Profile of Freshwater Fish, *Oreochromis Mossambicus* (Peters), *Kerala J. Aquat. Biol. Fish.* 5, 87-95.

Faheem, M. and Parvez Lone, K. 2017. Oxidative stress and histopathologic biomarkers of exposure to bisphenol-A in the freshwater fish, *Ctenopharyngodon idella*. *Brazilian J. Pharm. Sci.* 53(3): 1-9.

Faheem, M., Jahan, N. and Lone, K. P. 2016. Histopathological effects of Bisphenol-A on liver, kidneys and gills of Indian Major Carp, *Catla Catla* (Hamilton, 1822). *J. Anim. Plant Sci.* 26(2): 514-522.

Faheem, M., Khaliq, S. and Lone, K. P. 2019. Effect of Bisphenol-A on Serum Biochemistry and Liver Function in the Freshwater Fish, *Catla catla*, Pak. *Vet. J.* 39(1): 1-5.

Finney, D. J. (ed.). 1971. *Probit Analysis,* 3rd (ed.). Cambridge University Press, Cambridge, England. p. 333.

George, K. R., Gokul, G. N. and Malini, N. A. 2017a. Effects of different sub-lethal concentrations of plasticizer-Diethyl phthalate on Fresh water murrel, *Channa striatus* (Bloch). *J. Appl. Nat. Sci.* 9(1): 476-481.

George, K. R., Malini, N. A., Praveena, G. S. and Rejani, M. K. 2017b. Haematological and biochemical alterations in short term exposure to bisphenol a in *Oreochromis mossambicus* (Peters, 1852). *Pollut. Res.* 36(1): 57-62.

Giardina, B., Antonini, E. and Brunori, M. 1973. Hemoglobin in fishes: Structural and functional properties of trout hemoglobins. *Netherlands J. Sea Res.* 7 : 339-344.

Gingerich, W. H. 1982. Hepatic toxicology of fishes, p. 55-105. In: L.J. Weber (ed.). *Aquatic Toxicology*. Raven Press, New York.

Goodson, W. H., Luciani, M. G., Sayeed, S. A., Jaffee, I. M., Moore, D. H. and Dairkee, S. H. 2011. Activation of the mTOR pathway by low levels of xenoestrogens in breast epithelial cells from high-risk women. *Carcinogenesis*. 32 (11): 1724–1733.

Hastuti, S., Subandiyono, S. and Windarto, S. 2019. Blood performance of jaundice catfish *Clarias gariepinus*. *Aquac. Aquarium, Conserv. Legis. Int. J. Bioflux Soc.* 12(2): 480–489.

Hesser, E. F. 1960. Methods for Routine Fish Hematology. *The Progress. Fish-Culturist*. 22(4): 164–171.

Hinton, D. E. and Lauren, D. J. 1990. Integrative histopathological to detecting effects of environmental stressors on fishes. *Am. Fish. Soc. Symp.* 8: 51-66.

Hinton, D. E., Segner, H. and Braunbeck, T. 2017. Toxic responses of the liver, p 224-268. In : D. Schlenk and W. H. Benson (ed.). *Target Organ Toxicity in Marine and Freshwater Teleosts*. CRC Press, London.

Inadera, H., Sekiya, T., Yoshimura, T. and Matsushima, K. 2000. Molecular analysis of the inhibition of monocyte chemoattractant protein-1 gene expression by estrogens and xenoestrogens in MCF-7 cells. *Endocrinology*. 141(1): 50-59.

Jain, N. C. 1993. *Essentials of veterinary hematology*. Lea and Febiger, Philadelphia.

Kaliappan, K., Ganesan, S., Subramaniam, N., Mathan, R. and Vettaegounder, M. 2017. Sublethal concentration of bisphenol A induces hematological and biochemical responses in an Indian major carp *Labeo rohita. Ecohydrol. Hydrobiol.* 17(4) : 306–313.

Keum, Y.-H., Jee, J.-H., Lee, O.-H., Park, S.-I. and Kang, J.-C. 2005. In vivo effects of bisphenol A exposure on haematological parameters in Korean rockfish, *Sebastes schlegeli*. *J. Fish Pathol.* 18(3) : 293–301.

Kuhn, V., Diederich, L., Stevenson, K. I., Kramer, C. M., Lückstädt, W., Panknin, C., Suvorava, T., Isakson, B. E., Kelm, M. and Cortese-Krott, M. M. 2017. Red Blood Cell Function and Dysfunction: Redox Regulation, Nitric Oxide Metabolism, Anemia. *Antioxid. Redox Signal.* 26 (13) : 718–742.

Kumar, B. G. and Nandan S. B. 2014. Copper Toxicity: haematological and histopathological changes and prophylactic role of vitamin C in the fish, *Anabas testudineus* (Bloch, 1792). *J. Zool. Stud.* 1(3): 4–13.

Kwak, H. I., Bae, M. O., Lee, M. H., Lee, Y. S., Lee, B. J., Kang, K. S., Chae, C. H., Sung, H. J., Shin, J. S., Kim, J. H., Mar, W. C., Sheen, Y. Y. and Cho, M. H. 2001. Effects of nonylphenol, bisphenol A, and their mixture on the viviparous swordtail fish (*Xiphophorus helleri*). *Environ. Toxicol. Chem.* 20(4): 787–795.

Lee, C. C., Jiang, L. Y., Kuo, Y. L., Chen, C. Y., Hsieh, C.Y., Hung, C.F. and Tien, C. J. 2015. Characteristics of nonylphenol and bisphenol A

accumulation by fish and implications for ecological and human health. *Sci. Total Environ.* 502: 417-425.

Li, D., Bi, R., Chen, H., Mu, L., Zhang, L., Chen, Q., Xie, H., Luo, Y. and Xie, L. 2017. The acute toxicity of bisphenol A and lignin-derived bisphenol in algae, daphnids, and Japanese medaka. *Environ. Sci. Pollut. Res.* 24(30): 23872-23879.

Liu, Z. and Barrett, E. J. 2002. Human protein metabolism: its measurement and regulation. *Am. J. Physiol. Metab.* 283(6): E1105-E1112.

Lowry, O. H., Rosebrough, N. J., Farr, L. A. and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193(1): 265-275.

Macczak, A., Cyrkler, M., Bukowska, B. and Michalowicz, J. 2016. Eryptosis-inducing activity of bisphenol A and its analogs in human red blood cells (in vitro study). *J. Hazard. Mater.* 307: 328-335.

Makinwa, T. and Uadia, P. 2017. Occurrence of Bisphenol A (BPA) in Ponds, Rivers and Lagoons in South-Western Nigeria and Uptake in Cat Fish Evidence of Environmental Contamination. *Food Public Heal.* 7(1): 1-6.

Markey, C. M., Rubin, B. S., Soto, A. M. and Sonnenschein, C. 2002. Endocrine disruptors: from Wingspread to environmental developmental biology. *J. Steroid Biochem. Mol. Biol.* 83(1-5): 235-244.

Nalbandian, G. and Kovats, S. 2005. Understanding Sex Biases in Immunity: Effects of Estrogen on the Differentiation and Function of Antigen-Presenting Cells. *Immunol. Res.* 31(2): 91-106.

Negrin, A. 1969. Measurement of cholesterol in tissues with phospho-6-tungstic-12-molybdic acid. *Clin. Chem.* 15(9): 829-838.

Oehlmann, J., Schulte-Oehlmann, U., Kloas, W., Jagnytsch, O., Lutz, I., Kusk, K. O., Wollenberger, L., Santos, E. M., Paull, G. C., Van Look, K. J. W. and Tyler, C. R. 2009. A critical analysis of the biological impacts of plasticizers on wildlife. *Philos. Trans. R. Soc. B Biol. Sci.* 364(1526): 2047-2062.

Özaydin, T., Öznurlu, Y., Sur, E., Çelik, Ý. and Uluisik, D. 2018. The effects of bisphenol A on some plasma cytokine levels and distribution of $CD8^+$ and $CD4^+$ T lymphocytes in spleen, ileal Peyer's patch and bronchus associated lymphoid tissue in rats. *Acta Histochem.* 120(8): 728-733.

Pal, S., Sarkar, K., Nath, P. P., Mondal, M., Khatun, A. and Paul, G. 2017. Bisphenol S impairs blood functions and induces cardiovascular risks in rats. *Toxicol. Reports.* 4: 560-565.

Qiu, W., Zhao, Y., Yang, M., Farajzadeh, M., Pan, C. and Wayne, N. L. 2016. Actions of Bisphenol A and Bisphenol S on the Reproductive Neuroendocrine System During Early Development in Zebrafish. *Endocrinology.* 157(2): 636-647.

Research and Markets: Bisphenol-A - A Global Market Overview. 2016. [online] Available from: https://www.researchandmarkets.com/research/4xx8j9/bisphenola_a

Ribeiro-Varandas, E., Pereira, H., Monteiro, S., Neves, E., Brito, L., Ferreira, R., Viegas, W. and Delgado, M. 2014. Bisphenol A Disrupts Transcription and Decreases Viability in Aging Vascular Endothelial Cells. *Int. J. Mol. Sci.* 15(9) : 15791-15805.

Roberts, R. J. (ed). 2012. *Fish Pathology*, 4th ed. John Wiley & Sons Ltd., West Sussex.

Rogers, J. A. and Mirza, R. S. 2013. The effects of bisphenol- A on the immune system of wild yellow perch, *Perca flavescens*. *Water. Air. Soil Pollut.* 224(1728): 1-6.

Sakazaki, H., Ueno, H. and Nakamuro, K. 2002. Estrogen receptor α in mouse splenic lymphocytes: possible involvement in immunity. *Toxicol. Lett.* 133(2-3): 221-229.

Schnell, H. 1959. Linear Aromatic Polyesters of Carbonic Acid. *Ind. Eng. Chem.* 51(2): 157-160.

Segura, J. J., Jiménez-Rubio, A., Pulgar, R., Olea, N., Guerrero, J. M. and Calvo, J. R. 1999. In vitro effect of the resin component bisphenol A on substrate adherence capacity of macrophages. *J. Endod.* 25(5): 341-344.

Shakoori, A. R., Mughal, A. L. and Iqbal, M. J. 1996. Effects of Sublethal Doses of Fenvalerate (A Synthetic Pyrethroid) Administered Continuously for Four Weeks on the Blood, Liver, and Muscles of a Freshwater Fish, *Ctenopharyngodon idella. Bull. Environ. Contam. Toxicol.* 57(3): 487-494.

Sisodiya, M., Khare, M. and Kanhere, R. R. 2018. Hepatotoxic effects of bisphenol A on *H. fossilis*. *Trends Fish. Res.* 7(3) : 1-8 .

Sugita-Konishi, Y., Shimura, S., Nishikawa, T., Sunaga, F., Naito, H. and Suzuki, Y. 2003. Effect of Bisphenol A on non-specific immunodefenses against non-pathogenic *Escherichia coli*. *Toxicol. Lett.* 136(3): 217-227.

Tiwari, D. and Vanage, G. 2017. Bisphenol A Induces Oxidative Stress in Bone Marrow Cells, Lymphocytes, and Reproductive Organs of Holtzman Rats. *Int. J. Toxicol.* 36(2): 142-152 .

Wang, Q., Yang, H., Yang, M., Yu, Y., Yan, M., Zhou, L., Liu, X., Xiao, S., Yang, Y., Wang, Y., Zheng, L., Zhao, H. and Li, Y. 2019. Toxic effects of bisphenol A on goldfish gonad development and the possible pathway of BPA disturbance in female and male fish reproduction. *Chemosphere.* 221 : 235-245.

Weber, D. N., Hoffmann, R. G., Hoke, E. S. and Tanguay, R. L. 2015. Bisphenol A exposure during early development induces sex-specific changes in adult zebrafish social interactions. *J. Toxicol. Environ. Health. A.* 78(1) : 50-66.