Open access Journal International Journal of Emerging Trends in Science and Technology
Impact Factor: 2.838 DOI: http://dx.doi.org/10.18535/ijetst/v3i08.12

Effect of Bisphenol A on certain Hematological Parameters of *Heteropneustes fossilis*, Bloch

Authors
Aiswarya KS[1], Remya James[2]
[1,2] St. Joseph's College for Women, India, Kerala, Alappuzha
Email: *remya.james@rediffmail.com*

ABSTRACT
Effect of Bisphenol A on the hematological parameters total RBC count, total WBC count and hemoglobin content was assessed in Heteropneustes fossilis caught from wild. Behavioural changes were also monitored. It was found that 72 hour exposure of BPA at a concentration of 0.1g/L has lowered total RBC count and increased WBC count. Hemoglobin concentration in the blood also decreased exposure to BPA concentration of 0.1g/L. Severe mortalities in the experimental tanks were noticed at 0.5g/L and 0.1g/L concentrations of BPA. Another significant finding was that the specimens of experimental animal, Heteropneustes fossilis is extremely scarce to be obtained from wild in the regions of Alappuzha, Kerala.
Keywords- *Hetropneustes fossilis, Bisphenol A, total RBC count, total WBC count, hemoglobin*

INTRODUCTION

Bisphenol A is a synthetic chemical which is widely used in the production of polycarbonate plastics, epoxy resins and other plastic materials. Polycarbonate is widely used in food contact materials even in infant feeding bottles and this can lead to consumer exposure of BPA through food since the chemical can migrate from food contact materials (INFOSAN, 2009). The leaching of BPA from food contact materials increases with increase in temperature and age. Temperature seems to be a more influencing factor for the leaching of BPA into food, than the age of the food container (OEHHA, 2009; Rubin, 2011). BPA is known as an endocrine disruptor which acts as a weak environmental estrogen, but recent studies have shown that in stimulating certain cellular responses, it acts as a potent estradiol (Rubin, 2011).

Endocrine disruptors are naturally occurring or synthetic chemicals which mimic or interfere with the action of hormones in the body. Various studies in animals show that endocrine disruptors can have negative implications in the function of reproductive system as well as other functions of the body. It is advocated that similar effects are shown in human body leading to infertility and other reproductive problems. Evidences suggest that these chemicals may have effects on obesity and diabetes (NIH, 2007). The leaching of BPA into aquatic ecosystem mainly show its effects on the reproductive system of fishes and shell fish, which very well confirm its action as an endocrine disruptor by showing estrogen like activity (OEHHA, 2009).

Diethylphthalate, a kind of plasticizer, which also shows endocrine related effects, is found to affect various hematological parameters like red blood cell count, white blood cell count, hemoglobin content, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and mean cell volume in fresh water fish *Oreochromis mossambicus* (Sepperumal & Saminathan, 2013).

Heteropneustes fossilis is a fresh water swamp fish belonging to the family Heteropneustidae, easily available and is of cheap cost.

Though *H. fossilis* is a hardy fish capable of thriving various adverse environmental conditions, various studies have shown that many synthetic chemicals, mainly pesticides and insecticides can make it

vulnerable to toxic effects. Anoop *et. al.*, 2010 reported that dimethoate adversely affects behaviour of *H.fossilis* and a lower LD50 value was obtained for the chemical (Anoop *et. al.*, 2010)

Research has shown that BPA which is classified as an endocrine disrupting chemical stimulate the proliferation of macrophages in Carassius auratus at a dose of 0.005-50mg/L, while higher doses (500-1000mg/L) suppressed its proliferation in contrast with estradiol which was stimulative in all doses tested (Da-qiang *et. al.*, 2007). Studies in Zebra fish shows that hematopoietic stem cell formation is regulated in a temporally distinct manner by estrogen receptor 2 and it was found that estrogen-related compounds modify the expression of highly conserved transcription factor *RUNX1(AML1)* that is required for definitive HSC induction and is also the target of chromosomal alterations in leukemia (Carroll *et. al.*, 2011).

Studies on mice after injecting BPA into the abdominal cavity shows that it can destroy the male reproductive system of male mice like the action of estrogen. Due to the action of BPA sperm atogonia get detached from sertoli cells, get arranged in disorder, get displaced from basement membrane of seminiferous tubules and the chromatins of nuclei in sertoli cell and sperm antogonia gets flocculated (Yan-xia *et. al.*, 2008). Studies in the various cichlid species from Eleyele Resevoir, Southwestern Nigeria shows that the levels of polychlorinated biphenyls (PCBs) are higher than the threshold level of 0.023-0.047 ng/g recommended by United States Environmental Protection Agency and the concentration of heavier PCBs are higher than lighter PCBs in both sediment and fish tissue (Adeogun *et. al.*, 2016).

Exposure to BPA at concentrations of 0.8ppm, 1ppm and 5ppm have shown to cause negative histopathological effects on the liver of Hetropneustes fossilis including loosening of cells, pycnotic nuclei and fibrosis at respective concentrations (Roy *et. al.*, 2011).

METHODOLOGY

Fresh water cat fish, *Heteropneustes fossilis*, caught from wild, weighing 650 ± 2 g and length 29 ± 2 cm were collected from a fish farm. Fishes were acclimatized to the laboratory conditions for one week with constant supply of water and good lighting system. They were maintained in dechlorinated water. Water temperature and pH were monitored using standardized procedures. A single BPA dosage of 0.05g/L and 0.1g/ L of Bisphenol A was applied to experimental groups for 72 hours. Whole blood collected, adding EDTA, by severing the caudal vein, was freshly estimated for total WBC count, total RBC count and Hemoglobin concentration. Neubauer Hemocytometer was used for the total count estimations and Sahli's hemoglobinometer for hemoglobin estimation. Behavioral changes that were monitored were changes in opercular movement, body colour, feeding, body movement and mucous secretion

RESULTS AND DISCUSSION

The study proved that the chemical bisphenol A has the ability to kill the fresh water swamp fish *H. fossilis* at a concentration of 0.1g/L since only very few specimens were left from the experimental group after the application of endocrine disruptor BPA. LD50 could not be assessed because of the severe lack of wild specimens in the area.

The fishes left alive showed various behavioural changes that were close to fatal condition. After the application of a single dose of BPA at a concentration of 0.1g/L, at 72 hours, treated fish exhibited increased opercular movement and increased mucous secretion. Their movements slowed down and progressively became sluggish and lethargic. They also showed abnormal swimming movements and fading of their body colour. An outer layer of skin was seen withering from various regions of the body and feeding gradually decreased. The behavioural changes that were observed when Heteropneustes fossilis was exposed to organophosphate insecticide Dimethoate (Anoop *et. al.*, 2010) was very similar to the behavioural responses noted here.

The 0.1g/L dosage of BPA that was given to experimental groups which consisted of full grown fishes of almost 30cm length is very close to fatal concentration since very few fishes were found alive after 72hr exposure. From the present study it seems that the fresh water cat fish *H. fossilis* although a study fish, is susceptible to the lethal effects of bisphenol A.

Exposure of bisphenol A decreased the hemoglobin content of fishes. Hemoglobin content decreased from 11.7 gm% detected in the blood of control group fishes to 11.2 gm% in the experimental group treated with 0.1g/L of BPA.

BPA might have interfered with the synthesis of hemoglobin or might have caused the lysis of RBC or it may be binding with hemoglobin forming a complex, which can alter the secondary structure of hemoglobin which may affect the physiological functions of hemoglobin (Fang *et. al.*, 2011). Further detailed study and investigations should be carried out to prove the actual reason for the decrease in hemoglobin content. The decrease in hemoglobin concentration inturn leads to the decrease in the oxygen carrying capacity of the blood. Therefore decrease in the hemoglobin content will finally leads to oxygen stress resulting in various lethal effects in the body and death of the organism. The increased opercular movement may be caused by this oxygen deprivation and might have been carried out as an effort to increase the oxygen input.

After 72 hours of exposure of 0.1g/L BPA, in the live fishes of experimental group, it was found that the total RBC count has decreased and this can be explained as one of the reason for decrease in hemoglobin content of blood. The decrease in RBC count may be due to destruction of synthesized RBC or due to impaired synthesis of RBC. In vitro studies have linked BPA and its analogs with increased eryptosis of human red blood cells (Macczak *et. al.*, 2016). Oxidative stress and energy depletion can also elicit eryptosis (Foller *et. al.*, 2008). In the present study, total WBC count showed quite different pattern of change with the exposure of BPA when compared with changes in the erythrocyte levels. In the present study, WBCs were found to increase after 72 hour exposure of BPA. Diethylphthalate, a plasticizer which has shown to cause endocrine related effects, increased total RBC, WBC and hemoglobin when given to *Oreochromis mossambicus* at chronic concentration (Sepperumal & Saminathan, 2013). But studies in albino male rats after oral administration of BPA at a concentration of 150mg/kg has resulted in the decrease of red cell count, hemoglobin and packed cell volume (Ahmed *et. al.*, 2015).

White blood cells play a major role in the defence mechanism of the fish, and consist of granulocytes, monocytes, lymphocytes and thrombocytes. Granulocytes and monocytes function as phagocytes to salvage debris from injured tissue and lymphocytes produce antibodies. The increase in WBC count observed in the present study could be attributed to a stimulation of the immune system in response to tissue damage caused by BPA.

In the experimental set up with 0.05g/l concentration, any significant results could not be obtained since all the fishes died within 24 hours exposure. The procedure could not be repeated because of the scarcity of specimens.

Further studies in lower concentration need to be carried out for effectively finding out the effects of environmental exposure of these fishes in wild since the concentration under study is not normally detected in the aquatic bodies unless there is direct leakage of the chemical from factories or through some other source.

Table 1: Mean values obtained for the hematological parameters assessed in H. fossilis after 72 hour exposure to 0.1g/L BPA

Parameter	Control (n=10)	Experimental (n=10)
Hemoglobin	11.7 gm%	11.2 gm%
Total RBC	6590000 cells/cubic mm blood	4260000 cells/cubic mm blood
Total WBC	14100 cells/ cubic mm blood	29300 cells/cubic mm blood

Table 2: Behavioural changes that were noted in *H. fossilis* (n=10) after 72 hour exposure to 0.1g/L BPA

Opercular movement	Increased
Body colour	Faded
Feeding	Ceased
Body movement	Decreased
Mucous secretion	Increased
Skin texture	withered from various parts of the body
Mortality	8
Number of fishes alive after exposure	2

CONCLUSIONS

The study has shown that the chemical Bisphenol A at a concentration of 0.05g/l and 0.1g/l is toxic and fatal to the cat fish *Heteropneustes fossilis* which is said to have high surviving capacity to any environmental conditions when compared with other fishes.

ACKNOWLEDGEMENT

Authors are grateful to University Grants Commission for releasing the fund required for the study. We also express our sincere thanks to the management of St. Joseph's College for Women, Alappuzha for all the support and encouragement.

REFERENCES

1. Adeogun AO, Adedara IA, Farombi EO. Evidence of elevated levels of polychlorinated biphenyl congeners in commonly consumed fish from Eleyele Resevoir, Southwestern Nigeria. Toxicology and industrial health. 2016: 32(1): 22-29. doi: 10.1177/0748233713495585
2. Ahmed WMS, Moselhy WA, Nabil TM. Bisphenol A toxicity in adult male rates: Hematological, biochemical and histopathological approach. Global Veterinaria. 2015: 14(2): 228-238. DOI: 10.5829/idosi.gv.2015.14.02.9332
3. Anoop KS, Diwakar M, Shilpee S, Sunil KS, Ajai KS. Acute toxicity and behavioural responses of *Heteropneustes fossilis* to an organophosphate insecticide, Dimethoate. International Journal of Pharma and Bio Sciences. 2010: 1(4): 359-363
4. Carroll KJ, Dovey MC, Cutting CC, Sheward LV, Harris JM, Goessling W, North TE. 2011. Hematopoietic stem cell formation in Zebra fish is regulated in a temporally distinct manner by estrogen receptor 2. Blood. 2011: 118(21): 1268
5. Da-qiang YIN, Shuang-qing HU, Ying GU, Li WEI, Shu-shen LIU, Ai-qian ZHANG. Immunotoxicity of bisphenol A to *Carassius auratus* lymphocytes and macrophages following *in vitro* exposure. Journal of Environmental Sciences. 2007: 19(2): 232-237. doi:10.1016/S1001-0742(07)60038-2
6. Fang X, Cao S, Liu R. Interaction of Bisphenol A with Bovine Hemoglobin using Spectroscopic and Molecular Modeling Methods. Applied Spectroscopy. 2011: 65. DOI: 10.1366/11-06357
7. Foller M, Huber SM, Lang F. Erythrocyte programmed cell death. IUBMB Life. 2008: 60(10): 661-8. doi: 10.1002/iub.106.
8. International Food Safety Authorities Network. Bisphenol A (BPA) – Current state of knowledge and future actions by WHO and FAO. 2009. Available from http://www.who.int/foodsafety/publications/fs_management/No_05_Bisphenol_A_Nov09_en.pdf
9. Macczak A, Cyrkler M, Bukowska B, Michalowicz J. Eryptosis-inducing activity of bisphenol A and its analogs in human red blood cells (in vitro study). J. Hazard. Mater. 2016: 15(307): 328-35
10. NIH, National Institute of Environmental Health Sciences, Endocrine disruptors. 2007. Available from, http://www.niehs.nih.gov/-health/materials/endocrine_disruptors508.pdf
11. OEHHA, Office of Environmental Health Hazard Assessment, Toxicological Profile for Bisphenol A. 2009. available from, http://www.opc.ca.gov/webmaster/ftp/projec

t_pages/MarineDebris_OEHHA_ToxProfiles/Bisphenol%20A%20Final.pdf

12. Roy S, Kalita JC, Mazumdari M. Histopathological effects of Bisphenol A on Liver of *Heteropneustes fossilis* (Bloch). The Ecosan. 2011: 1: 187-190
13. Rubin BS. Bisphenol A: an endocrine disruptor with widespread exposure and multiple effects. J Steroid Biochem Mol Biol. 2011: 127(1-2): 27-34
14. Sepperumal U, Saminathan S. Effect of diethylphthalate on the parameters of the freshwater fish *Oreochromis mossambicus* (Tilapia). European Journal of Zoological Research. 2013: 2(4): 55-59
15. Yan-xia S, Ji-fang L. The damnification of bisphenol A by lipid peroxide and pathologic change on male mice test. Chinese journal of laboratory diagnosis. 2008. Available from: http://en.cnki.com.cn/Article_en/CJFDTOTAL-ZSZD200802020.htm