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The Assessment of the Toxic Effects of Bisphenol A (BPA) on Physiology and Reproduction of the Fish Species *Oreochromis mossambicus*

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The industrialized world produces massive amounts of plastic every year. Recent research information suggests that BPA exhibits hormone-like properties, raising concerns about its suitability in some consumer products and food containers. The most important human exposure route to BPA is diet, which includes consumption of unclean food and water. Exposure to BPA has resulted in adverse effects on reproduction, embryonic development, and the onset of genetic abnormalities in both aquatic and terrestrial animals. However, reports of the effects of BPA on reproductive physiology in fish are limited. This study was aimed to study the adverse effect of BPA on the regulation of reproductive genes in Mozambique tilapia (*Oreochromis mossambicus*) fish species. The tissue distribution patterns of GnRH1 and GnRH1 in adult *Oreochromis mossambicus* are shown in predominantly expressed in brain tissues but after the exposure of BPA the gene

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expression were reported down regulated. When t test was applied on the obtained data we have reported the statically significant down regulation of the GnRH2 (t test P = 0.0395) after the exposure of BPA 5mg BPA/L. This study showed the adverse effect on the reproductive genes of the *Oreochromis mossambicus*.

Keywords: Oreochromis mossambicus; tilapia fish; bisphenol A; GnRH1; GnRH1.

1. INTRODUCTION

“Synthetic chemicals known as endocrine disruptors were first created for a specific purpose, such as a pesticide, plasticizer, or solvent, but it has now been shown that they also have a number of adverse impacts” [1]. Endocrine disruptor has been defined as "an external chemical or mixture that affects endocrine system function [s] and subsequently causes deleterious effects in an intact organism, its progeny, or subpopulations" [2]. “Endocrine-disrupting chemicals [EDCs] work by simulating endogenous hormones, opposing natural hormones, affecting the pattern of hormone synthesis or metabolism naturally, changing the way hormones travel through the body or changing the amounts of hormone receptors” [3]. “EDCs have the potential to obstruct typical growth and reproduction, which are regulated by a variety of hormonal signals, as a result of these effects. EDCs were initially believed to only act through nuclear hormone receptors, such as retinoid receptors, thyroid receptors, androgen receptors, progesterone receptors, and oestrogen receptors [ERs]” [4]. “Recent research indicates that the methods through which EDCs act, however, are far broader than previously thought. Indeed, research has demonstrated that EDCs can affect nuclear receptor signalling in addition acting through a variety of other mechanisms that affect the endocrine and reproductive systems, including nonsteroid receptors, transcriptional coactivators, enzymatic pathways involved in steroid biosynthesis and/or metabolism, and numerous others” [4,5]. Direct impacts on genes [6] and their epigenetic impact [7] are two other, less well-known methods by which EDCs work. These consequences are especially concerning since changes in genetic programming at early stages of development may have lasting impacts years later and may also result in the transmission of disease from one generation to the next [8].

The aquatic environment has been referred to as "the ultimate sink" for both natural and man-made chemicals. Since EDCs have been discovered in freshwater, estuarine, and marine environments, it is possible that EDCs have an impact on the aquatic organisms that live in these environments. Others [9,10,2] have examined the evidence for the presence of endocrine disruption in wildlife in general and specifically in marine and estuarine creatures [11]. Studies on the effects of EDCs on the reproductive axis have primarily concentrated on peripheral reproductive processes including vitellogenesis and spermatogenesis [12]. Nevertheless, the brain-pituitary-gonadal [BPG] axis's three hierarchies are all engaged in the complicated mechanisms by which EDCs exert their effects. Recent data support the idea that EDCs may disrupt the neuroendocrine system's controls on reproduction [13].

“A common EDC which is an industrial synthetic chemical used in the manufacturing of polycarbonate plastics and epoxyresins is bisphenol A [BPA]” [14]. “Additionally, BPA is utilised in the manufacture of polysulfone, polyester resins, thermal paper, baby bottles, medical equipment, food packaging, and a variety of coatings, including marine, protective, powder, can, and coil coatings” [15,16]. “BPA exposure to aquatic environments from petrochemical industry discharge, urban sewage, and landfill leachates is ongoing, which poses a major threat to the health of aquatic creatures” [17]. “Since its toxicity can alter fish population, behaviour, and central nervous system, its negative impacts on aquatic creatures have also caused great worry” [18]. Furthermore, according to numerous studies [19-22], BPA can have a considerable impact on fish's growth, morphology, biochemical variables, and histological structure. Fish are primarily employed to monitor pollution in the aquatic environment because they play a significant part in the trophic web and have the ability to bioaccumulate hazardous compounds like BPA even at low concentrations [23]. Numerous studies have shown that BPA toxicity has a negative impact on the oxidative defence system and reproductive dysfunctions in many fish species, including the Japanese medaka,

Oryzias latipes [Li et al., 2016; Minghong, 2011], swordtail, *Xiphophorus helleri* [Kwak et al., 2001], rainbow trout*, Oncorhynchus mykiss* [Bjerregaard, 2007], and yellowfin seabream, *Acanthopagrus latus* [22]. "The gonadotropin-releasing hormone [GnRH] neurosecretory system is one of these networks that is crucial in regulating the activity of the reproductive axis in vertebrates" [24]. "In the GnRH neurons of the hypothalamus, GnRH decapeptide is generated. Follicle-stimulating hormone [FSH] and luteinizing hormone [LH], which act on the gonads to stimulate gonadal maturation, gametogenesis, and steroidogenesis, are produced when GnRH binds to GnRH receptors [GnRHR] on the cell surface of pituitary gonadotropes in the anterior pituitary with specific high affinity. Additionally, the GnRH system in teleost species was studied. GnRH-1 and GnRH-2 levels in mature men and females of striped bass increased during gonad recrudescence and peaked around the time of spawning" [25]. In European sea bass, the mRNA levels of GnRHs and GnRH receptors rose during sexual development [6]. A number of internal and external stimuli, particularly sex hormones, fine-tune the activity of the GnRH neurons, making them a possible primary target for EDCs. Fish are the foundation of a substantial commercial fisheries and aquaculture business, in addition to having significant recreational value. Commercial and recreational fisheries may be harmed if Bisphenol in the aquatic environment are endangering fish population sustainability over time by altering fish reproduction success.

2. METHODOLOGY

Fish rearing conditions and Bisphenol treatment: Samples of Mozambique Tilapia (*Oreochromis mossambicus*) were obtained from Narmada River of M.P., The fishes were fed with optimum fish food twice daily. After acclimatization of 30 days, Fish were exposed to BPA in specific different concentration (5mg BPA/L) after determining LC_{50}, 3 sub-lethal doses were selected. After 7 days fish brain tissues were dissected and then frozen using liquid nitrogen and kept individually at −20 °C, within couple of days RNA were extracted.

RNA extraction and reverse transcription (RT): Tissue samples were homogenized in TRIZOL (Invitrogen) and total RNAs were isolated as previous described (Meng L et al. 2010). The concentration and purity of isolated RNA were assessed by the spectrophotometric method with The Qubit RNA HS Assay Kit (Invitrogen) We also checked the RNA integrity by analyzing 18S and 28S ribosomal RNA (rRNA) ratios with 1% agarose gel eletrophoresis. The total RNAs were further treated with RNase-free DNase I (Promega, USA) to remove genomic contamination. The cDNAs were synthesized from 1000 ng total RNA with iScript™ cDNA Synthesis Kit (BioRAD) (Wang et al., 2010).

Fig. 1. The fish species *Oreochromis mossambicus,* selected as experimental model in present study

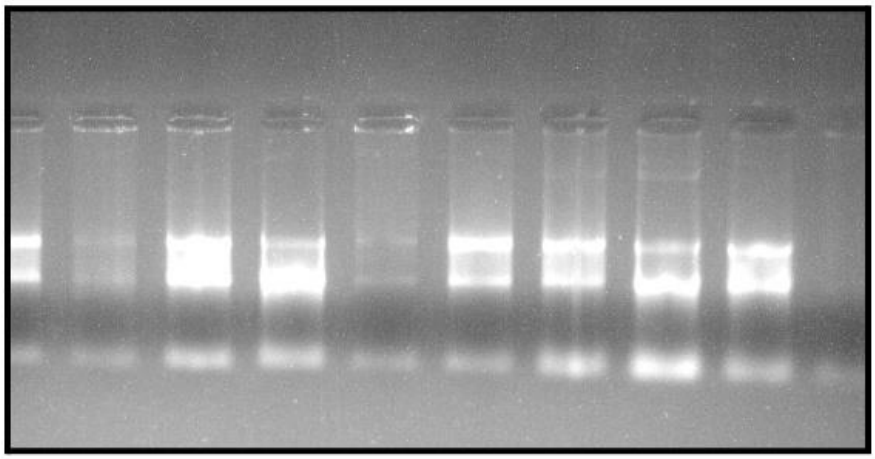

Fig. 2. Representative image of good quality RNA extraction using trizol method from fish tissue (Brain)

Quantitative real-time PCR (qRT-PCR): qRT-PCR was performed using SYBR Green ExTaq II kit (TaKaRa) and AriaMx Real-time PCR System (Agilent). The PCR reaction was carried out in a final 20 µL volume, with SYBR Green Premix Ex Taq ™, 0.4 µM of each forward and reverse primer, 2.5 µL RT reaction solution. Each sample was analyzed in triplicate using the following protocol: 95 °C/30 s; 40 cycles of 95 °C/5 s; 60 °C/30 s. Agilent area1.6 software was used to analysed the density of SYBR green I and to determine the quantification cycle (Cq) value. The delta CT) method was applied for the conversion of the Cq value into Fold change.

Primer used in the present study:-

GnRH1Reverse5′TGCGTCCATTTCCTCTGTC AGTGT 3′
GnRH1Forward5′TCCAGGAGGAAAGAGGGG TCTGGA3’

(Gal & Gad, 2012)

GnRH-R2-F5′-CGTGCGGTGAAGGCGAAGG GGGTGG-3′

GnRH-R2-R5′-ACACAACCCCAACmTAAGCA AGCATA-3′

(Duan et al. [26])

18sRNA primers (Housekeeping gene)
18sRNA-R 5′- TGATTGGGACTGGGGATTGAA-3′
18sRNA-F5′-TAGCGACGGGCGG TGTGT-3′

(Duan et al. [26])

3. RESULTS AND DISCUSSION

The hypothalamic neurons produces GnRH and is secreted into the hypophyseal portal circulation to act primarily on the anterior pituitary. The GnRH binds to the gonadotropin-releasing hormone receptor (GnRHR), on the cell surface of a specific pituitary cell type, the gonadotrope cells, and which starts the downstream signalling for the production of these gonadotropins. Hence in the present study the expression of the gonadotropin-releasing hormone receptor 1 &2 were studied in *Oreochromis mossambicus* upon the treatment of Bisphenol A (BPA) [27].

Table 1. The t and F test results on the mRNA of GnRH1 gene in brain tissues between control and BPA treated fish

Table Analyzed (GnRH1)	Data 1
Column A	CT
Column B	BPA (5mg/L)
Unpaired t test	
P value	0.4312
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.8289 df=8

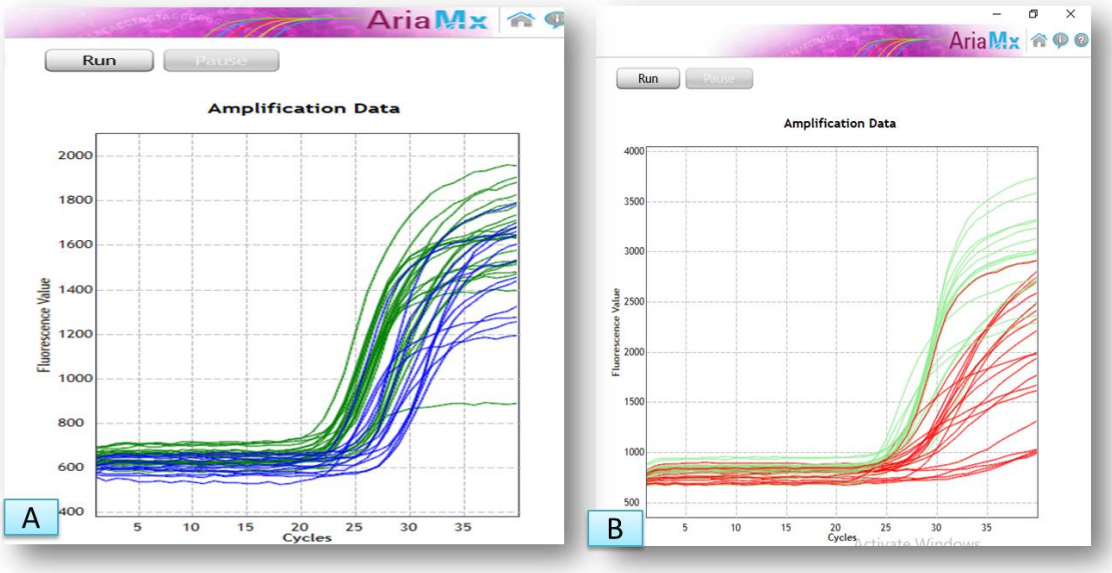

Fig. 3. The image of the amplification plot of the studied genes GnRHR1A (A: blue peaks), GnRHR1B (B: Red peaks)] Housekeeping gene (Green Peaks)

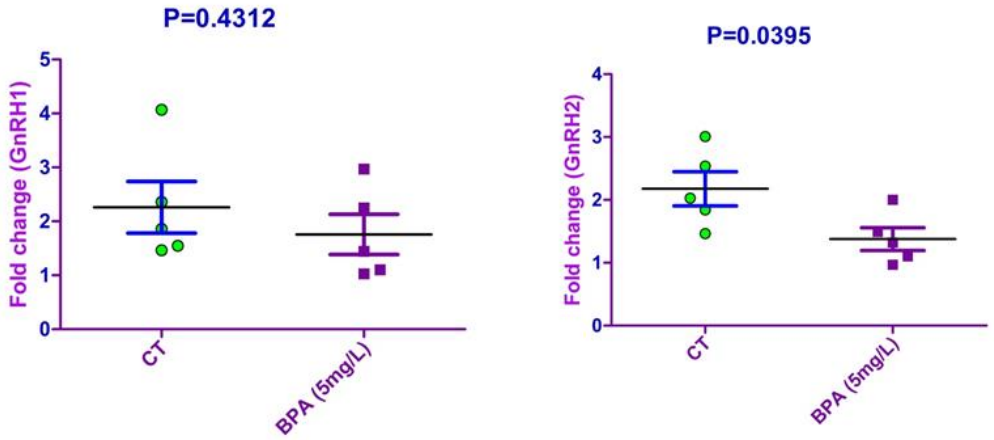

Fig. 4. The t test results on the mRNA of GnRH1 and GnRH2 gene expression in brain tissues between control and BPA treated fish

Table 2. The t and F test results on the mRNA of GnRH2 gene in brain tissues between control and BPA treated fish

Table Analyzed (GnRH2)	Data 1
Column A	CT
vs	vs
Column B	BPA (5mg/L)
Unpaired t test	
P value	0.0395
P value summary	*
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=2.457 df=8

The qRT-PCR analysis was successfully done as the graph lines showed in the Fig. 3 showing the mRNA expression profiles of GnRHR1 (A)and GnRHR2 (B) in brain tissues after BPA exposure. The tissue distribution patterns of GnRH1 and GnRH2 in adult *Oreochromis mossambicus* are shown in Figs. 3 and 4. GnRH1 and GnRH2 were predominantly expressed in brain tissues but the after the exposure of BPA the gene expression were reported down regulate as indicated in the Fig. 4. When t test was applied on the obtained data we have reported the statically significant down regulation of the GnRH2 (Table 1, P = 0.0395). Slight different pattern was fund due different dosing of BPA in the earlier study, In a study by Zohar et al., 2010, the 35-day BPA exposure at environmental concentrations did not affect mRNA transcript of brain GnRH2 in *G. rarus*, suggesting the less concentration can be tolerate but high concentration can be affect the gene expression.

4. CONCLUSION

The tissue distribution patterns of GnRH1 and GnRH1 in adult *Oreochromis mossambicus* are shown in predominantly expressed in brain tissues but the after the exposure of BPA the gene expression were reported down regulated.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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