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Effects of Bisphenol A on Haematological, Gonadal Gene Expression and Histopathological Biomarkers in Genetically Improved Farmed Tilapia

Shyam Lal Tudu[1], P. Sriram*[1], Uma Arumugam[2], Nallaiah Hemamalini[1] and S.A. Shanmugam[1]

Tamil Nadu Dr. J. Jayalalithaa Fisheries University
Vaniyanchavadi, Chennai, Tamil Nadu, India- 603103

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Abstract

Bisphenol A (BPA), a common aquatic contaminant, was tested on Genetically Improved Farmed Tilapia (GIFT) juveniles. The 96-h median lethal concentration was 6.794 mg/L. Sublethal exposure (4–6 mg/L for 7 days) altered haematology, histopathology, and gonadal defense gene expression. Leukocyte counts rose significantly at 4 mg/L, while erythrocyte counts decreased across treatments. MCV, MCH, and MCHC values increased. Histopathological damage was evident in gills, gonads, brain, and kidney. Defense genes GST, OP450, and p53 were upregulated in gonads. Overall, BPA exposure significantly disrupted blood parameters, tissue integrity, and cellular defense mechanisms in GIFT juveniles.

Keywords: Bisphenol A; GIFT; Histopathology; Gonadal gene expression

Introduction

Endocrine-disrupting chemicals (EDCs) like PCBs, DDT, BPA, dioxin, atrazine, phthalates, perchlorate, mercury, organophosphate insecticide, etc., are substances made available in the environment, food sources, personal care products, and manufacturing product those interfere with the normal functioning of the endocrine system of an organism (Ankley *et al*. 2009). In the aquatic environment, the ability of an EDC to affect an individual or population would depend on many factors, including the potency or efficacy of the EDC, its concentration, duration of exposure, bioconcentration, life stage exposed, season, or other environmental stressors like temperature, salinity and other contaminants present and the mobility of the individual (Pait and Nelson 2002).

Bisphenol A (BPA, 4,4'- propane-2,2-diyl) diphenol is an organic synthetic compound and estrogenic endocrine disrupting chemical (Faheem *et al.* 2016). It is a commercially used chemical additive in polycarbonate plastic production to manufacture thermal paper epoxy resins (Staples *et al.* 1998). It is one of the highest volume chemicals produced worldwide and its demand is increasing due to the ever-increasing demand and production of plastic products (Talpade *et al.* 2018). Numerous studies found that BPA leaches from polycarbonate baby bottles (Vandenberg *et al*. 2007) and reusable water bottles (Le *et al.* 2008). BPA is one of the major flame retardants (Meerts *et al.* 2001) and it has recently been shown also to antagonize thyroid hormones (Moriyama *et al.* 2002) and androgen action (Lee *et al*. 2003). The endocrine-disrupting effect of BPA was first reported by Krishnan *et al.* (1993). This chemical has been concerned with animal development and humans affecting the reproductive system since being a

*Corresponding author E-mail:dr.psriram@gmail.com
[1]Institute of Fisheries Post Graduate Studies, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Vaniyanchavadi, Chennai, Tamil Nadu, India- 603103.
[2]Directorate of Incubation and Vocational Training in Aquaculture, ECR - Muttukadu, Kancheepuram - 603 112.

well-known estrogenic endocrine-disrupting agent (Yokota et al. 2000; Kang *et al.* 2002). A study revealed that BPA exposure induced oxidative stress and reproductive toxicity in *Anabas testudineus* (Devi *et al.* 2015). It is estimated that BPA in the environment is mostly present in water and suspended solids (52%), soil (25%) and sediments (23%) (Staples *et al.* 1998; Cousins et al. 2002). BPA was found at a concentration in the range of several tens to several hundreds of nanograms per litre in most of the rivers surveyed and some of the highest concentrations (54–1950 ng/L) were found in rivers in Chennai, India (Yamazaki *et al.* 2015).

With this background, the present study was focused on assessing the endocrine disrupting effect of bisphenol A in Genetically improved farmed Tilapia (GIFT) based on the haematological, histopathological changes and gene expression profile in various tissues of GIFT.

Materials and Methods

Experimental Fish

GIFT juveniles weighing 15g and an average length of 8.5 ± 2cm were collected from the fish farm in Chennai and transported to the laboratory in plastic packages filled with oxygenated water. The fishes were acclimatized to the laboratory conditions before the experiment (for 14 days), kept in aquarium tanks of 80L capacity, maintained at normal temperature and fed with commercial pelleted feed. The fish were maintained in well-aerated water with salinity of 5 ppt and pH of 7.5 - 8.5.

Determination of Lethal Concentration 50 (LC50)

21 tanks with a capacity of 35L were used in the study. In each tank six fish were maintained. Different concentrations of BPA were added to the fish tanks to obtain final concentrations of 7 mg/L, 9 mg/L, 11 mg/L, 13 mg/L, 15 mg/L, 17 mg/L and 19 mg/L. Three tanks without BPA were treated as control. The experiment was conducted in triplicate and the fish were exposed for 72 hours. The observations were recorded every 30 minutes for the first 12hr and later every 2hr. After 96 h, the LC50 values were determined by probit analysis. Based on the LC50 value, the sub-lethal concentration of BPA was determined.

Histopathology

Gill, kidney, gonad, and brain tissue from control and BPA-exposed Tilapia were collected to study histopathological changes. The tissue samples were treated with various concentrations of ethanol. The sections were stained with haematoxylin and eosin and examined under a high power microscope (200X).

Haematological parameters

After the completion of blood collection, the haematological parameters, including hematocrit, RBC, WBC, haemoglobin, MCH, MCV and MCHC, were assessed.

Haematocrit assessment was carried out using a microhematocrit method. The blood was collected in the heparinized tubes, and the samples were centrifuged at 12,000 rpm for 5 min. The haematocrit values were read on a microhaematocrit reader. The Neubauer hemocytometer was used to measure RBC count following Blaxhall and Daisley (1973).

$$\text{RBC (106/ml)} = \frac{\text{C X D x 100 x 4000}}{\text{S x 80}}$$

Where C -Number of cells counted, D - Diluting factor and S - Number of 1mm square counted

The cyanate-haemoglobin method was used, as described by Larsen and Snieszko (1961).

The blood secondary indices, including MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin), and MCHC (mean corpuscular haemoglobin concentration), were calculated by the following terms (Bain *et al.* 2016).

$$MCV\ (dL) = \frac{\text{Packed cell volume /dL x 10}}{\text{RBC/µB (in } 10^6\text{)}}$$

$$MCH\ (pg) = \frac{\text{Haemoglobin/dL x 10}}{\text{RBC/µL (in } 10^6\text{)}}$$

$$MCHC\ (g/dL) = \frac{\text{Hb/dL x 100}}{}$$

Gene expression profiling

Total RNA was extracted from the collected gonad tissues using an RNA iso-plus kit (Takara Bio Inc. Japan) according to the manufacturer's protocol. First-strand cDNA was synthesized from 2 µg of total RNA using the first-standard cDNA synthesis kit (Thermo Scientific, USA). The relative gene expression study was carried out and β-actin transcript was used as an internal control. The quantitative real-time polymerase chain reaction (qRTPCR) was carried out in a C1000 Touch thermal cycler-CFX96 Real-time PCR (Bio-Rad, USA). The list of primers used for the gene expression study is given in Table 1. qRT-PCR was performed using 20 ng of cDNA template, 10 µM of each primer (forward and reverse) and 1x SYBR Green PCR Master Mix Kit (Takara Bio Inc. Japan) and nuclease-free water to make a total volume of 20 µl. The qRT-PCR cycle threshold (Ct) values were measured and a relative expression level of the specific gene was presented as 2-ΔΔCt.

Results

Determination of LC50

Tilapia fingerlings were exposed to different concentrations of BPA ranging from 7 to 9 mg/l for 96 h. Fishes were observed for mortality, behaviour and condition of the fin, scale, mouth, and eyes for 96 h and the results were given in Table 2 and Fig. 1. No mortality of fishes was observed in the control group. BPA at 7 mg/l caused no mortality at the end of 24 h and; however, as the time increased, high mortality was observed. At 17 and 19 mg/L, 90% of mortality was observed within 24 h and 100% mortality was noticed within 72 h. Tilapia fingerling showed irritation movement and jumping when exposed to BPA at all concentrations. As time advances, complete loss of movement, inability to swim, increased mucus secretion, and change of colour of eyes were observed in all the treatment groups.

The median lethal concentrations (LC_{50}) of BPA were calculated using SPSS software (version 2.0) by probit-regression method. The LC_{50} with 95% confidence intervals at the end of 24 h, 48 h, 72 h and 96 h were given in Table 3. The LC50 of Bisphenol A for 96 h was found to be 6.794 mg/l.

Histopathology

Gill, kidney, gonad, and brain tissue from normal and BPA exposed Tilapia fingerlings were analyzed for histopathological changes.

Gill

The primary and secondary gill lamellae of control fish were intact with normal epithelial cells. Gills from the control group showed chondrocyte skeleton primary lamellae and

parallel thread-like secondary lamellae made up of pillar cells and protected by mucus cells (Fig. 2A). Several undifferentiated basal cells were also noted in the gills of fish from the control group. Fingerlings exposed to BPA revealed extensive damage in their gill architecture with mild congestion and fusion of villi in fishes treated with 4 mg/l of BPA (Fig. 2B). Congestion, shortening and fusion of laminae were observed in the fish treated with 5 mg/l of BPA (Fig. 2C) whereas congestion of gills was observed in fishes exposed to 6 mg/l of BPA (Fig. 2D).

Gonad

Fishes in the control group showed no histopathological changes in the ovary with normal nucleus, previtellogenesis and stroma (Fig. 3A). Fishes treated with BPA showed histopathological changes in ovaries at all concentrations. Fishes exposed to 4 and 5 mg/L of BPA showed interfollicular lining epithelial hyperplasia (Fig. 3B) and 3C), whereas degeneration interfollicular lining epithelial hyperplasia was observed in the ovary of fishes exposed to 6 mg/l of BPA (Fig. 3D).

Kidney

The kidneys control group of Tilapia showed normal, slightly spherical glomeruli with proper Bowman's space brush border of proximal tubules and lumen of distal tubules (Fig. 4A). The kidney of fishes treated with 4 mg/L BPA showed congestion, focal haemorrhage, tubular degeneration and focal necrosis whereas edema, tubular epithelial degeneration and denudation into the lumen were observed in fishes exposed to 5 mg/l of BPA (Fig. 4B and 4C). Fishes exposed to high concentrations (6 mg/L) of BPA, congestion, edema, tubular epithelial degeneration, denudation into the lumen and hyaline casts were observed (Fig. 4D).

Brain

Histopathology of the brain from the fishes maintained in control group showed no histological changes with intensely stained dark neurons in the cerebral cortex, shrunken basophilic cell bodies and proximal dendrites (Fig. 5A). Histopathology of the brain revealed meningitis, focal necrosis and gliosis at 4 mg/l of BPA (Fig. 5B) whereas, congestion, mononuclear cell infiltration and focal necrosis were observed in treatment group 5 mg/L (Fig. 5C). Fishes treated with BPA at 6 mg/L showed congestion in the brain with mononuclear cell infiltration, degeneration, neuronal vacuolation, haemorrhage and hemosiderin pigment (Fig. 5D).

Effect of BPA on haematological parameters

The haematological parameters in the control and treatment groups are given in Table 4. There was no significant difference in the haemoglobin concentration and haematocrit between the treatment and control groups. There was a significant increase ($P<0.05$) in total Leukocyte count in fishes exposed at 4 mg/l compared to control and other treatment groups. However, there was no significant difference in the total leukocyte count in the treatment groups at 5 and 6 mg/L compared to control. The erythrocyte count was significantly ($P<0.05$) reduced in all the treatment groups compared to control. Increased MCV, MCH and MCHC were observed in treatment groups compared to control and it was statistically significant ($P<0.05$) at 4 and 6 mg/L.

Gene expression

The expression of the GST gene in gonad tissue of tilapia fingerlings were significantly altered by BPA treatment and it showed a dose-dependent increase in treatment groups, which

differed significantly from other treatment groups and the control fishes. The expression of GST was high in fishes treated with 6 mg/l of BPA, and it differed from that of other treatment groups and the control group.

The expression of the OP450 gene in gonads was altered with BPA treatment, and it was significantly higher in the treatment group than in the control group. The expression of OP 450 was higher in fishes exposed to 5 mg/L, followed by 4 mg/L and 6 mg/L. The OP 450 expression in treatment groups showed no dose relation, and the results differed significantly from each other.

Similarly, the expression of the gonadal P53 gene was altered by BPA treatment, and it was significantly higher in all treatment groups than in the control group. Among the treatment groups, the expression of the P53 gene was least in the high dose group (6 mg/L), and it differed significantly from fishes treated with 5 mg/L, which showed no significant difference from those treated with 4 mg/L. Fishes treated with BPA at 5 mg/L showed the highest level of P53 gene expression and it differed significantly from control and those treated with 6 mg/L. The expression of gonadal genes in BPA exposed fish is provided in Table 5 & Fig. 2.

Discussion

BPA is one of the highest produced chemicals in the world, with a total production of over 3.9 million metric tons (Tsai 2006). More than 90% of BPA is used in the production of epoxy resins and polycarbonate plastics (Vandenberg *et al*. 2009; Goodman *et al*. 2017). Oreochromis niloticus, a freshwater fish, was used as an experimental model in the present study and the fingerlings were exposed to Bisphenol A to study its effect on haematological and gonadal genes expression.

The acute toxicity effect of a chemical was determined by studying the effects of a chemical after high level exposure to a chemical toxic for a maximum of 96 h. The toxicity of a chemical is generally expressed as median lethal concentration (LC50), the concentration of a chemical that causes the death of 50% of the population or the test organism within 96 hours (Libralato *et al*. 2016). In the present study, the LC50-96 h value of the BPA in O. niloticus was 6.794 mg/l. In harmony with our findings, Faheem and Lone (2018) reported a 96h LC50 value of 6.323 ppm BPA in Ctenopharyngodon idella. Iqbal *et al.* (2004) studied acute toxicity of nitrate in common carp (Cyprinus carpio) and reported LC50 values of 995 and 865 ppm after 48 and 96 h, respectively and these values were high compared to the LC50 of BPA. Similarly, 96 hr LC50 value of zinc in Labeo rohita fingerlings was reported as 156 ppm (Loganathan *et al.* 2006). The LC50 value of cypermethrin in Clarias gariepinus was reported as 0.063 mg/l after 96 h of exposure (Ayoola and Ajani 2008). Moustafa *et al.* (2007) studied the acute toxicity of phenol in Nile tilapia exposed to different concentrations for 72 hours and reported an LC50 value of 25.0 mg/L.

Histopathology of tissues

Biomarkers are biological responses to any contaminants and indicate the change in the status of the organism.

Gills are one of the organs most commonly affected by waterborne pollutants since they are constantly in contact with the water and have a high adaptation capacity. The most commonly recorded response to the environmental pollutants in fishes is alterations in the gills (Mallatt 1985). Histological alterations observed in fish gills are considered a fast and valid method to determine the damage caused by exposure to different pollutants in

fish (Arellano *et al*. 2001). In the present study, fishes exposed to Bisphenol A showed several histological alterations like mild congestion, fusion, shortening of primary laminae, fusion of secondary laminae and increased primary laminae tip length. Elshaer *et al.* (2013) reported cellular necrosis and degeneration of secondary lamellar epithelium in Gambusia affinis and Poecilia reticulata exposed to 50 µg/L BPA for 15 days and necrosis in the secondary gill lamellae was observed after 30 days of treatment in Gambusia affinis and Poecilia reticulata. Tietge *et al*. (1988) reported hyperplasia, fusion and inspiration of auxiliary lamellae and degeneration of essential lamellae in the gills of fishes exposed to BPA. Mazon *et al.* (2002) reported epithelial lifting, rupture, peeling of lamellar epithelium, lamellar fusion, hyperplasia and cellular hypertrophy in tropical freshwater fish gills, Prochilodus scrofa exposed to copper. Gill degeneration and putrefaction reflect the coordinated impact of poisons on angle wellbeing (Figueiredo-Fernandes *et al.* 2007).

The kidney is the major hematopoietic and osmoregulatory organ in the fish. Apart from their role in the removal of wastes from the blood and, it is also responsible for selective reabsorption, which helps in maintaining the volume and pH of blood and body fluids, erythropoiesis and regulation of blood pressure (Iqbal *et al*. 2004). In our study, congestion, focal haemorrhage, tubular degeneration and focal necrosis were noticed in the kidneys of O. niloticus exposed to low concentrations of BPA, whereas edema tubular epithelial degeneration and denudation and hyaline casts were noticed in fishes exposed to the high concentration of BPA

Gupta and Srivastava (2006) reported dilation, oedema and hypertrophied nuclei of renal tubules, glomerular vacuolization, necrosis and pyknotic nuclei in mesenchymal tissues were observed in the kidney of Channa punctatus following exposure to sublethal concentration of zinc. Faheem and Lone (2018) reported damaged renal tubules, shrinkage of tubules and tubule lumen and degeneration of tubules and hematopoietic tissue in kidneys of Ctenopharyngodon idella exposed to sublethal concentration of BPA.

In the present study, BPA effect on the fish brain showed meningitis, focal necrosis and gliosis, congestion, MNC infiltration, focal necrosis, neuronal vacuolation, haemorrhage and hemosiderin pigment were noticed in the brain of O. niloticus exposed to BPA. Loganathan *et al.* (2006) studied histopathological changes in the brain of Labeo rohita fingerlings exposed to zinc (5 and 10 ppm) for a duration of 5 and 15 days. They reported swelling of pyramidal cells with binucleated nuclei at 5 ppm, whereas severe necrosis of cerebral neuronal cells, mild vacuolar changes with empty spaces at 10 ppm.

The brain tissue of Nile tilapia, O. niloticus, exposed to phenol revealed blockage of cerebral and meningeal veins, perivascular edema and melanomacrophages collection, submeningeal drain with diffuse gliosis and neuronal degeneration, diffuse malacia with satellitosis and neuronophagia (Moustafa *et al.* 2007). In the present study, interfollicular lining epithelial hyperplasia and degeneration were observed in the ovaries of fishes exposed to BPA. In agreement with our findings, Yön Ertuğ and Akbulut (2014) reported severe deterioration and dose-dependent structural deformities, decreased number of primary oocytes and an increased number of atretic oocytes in the ovaries of Zebra fish exposed to BPA at 3 mg/l and 5 mg/l of BPA for 7 days. Maqbool and Ahmed (2013) studied the effect of

monocrotophos exposure to freshwater murrel, Channa punctatus to 1 ml/l and 2 ml/l for 15 and 45 days and reported decreased number of vitellogenic oocytes and atresia in the ovaries of fishes exposed to monocrotophos. Ram and Sathyanesan (1987) studied the long term effect of commercial cythion containing 50% (w/w) malathion in Teleost Fish, Channa punctatus. They reported increased oocyte degeneration resulting in inhibition of gonadal development and lower gonadosomatic index in fishes exposed to cythion at 20 mg/L for 6 months.

Haematological parameters

Haematological parameters can be used as bioindicators in fish and target organs of toxicity following exposure to xenobiotics such as pesticides and metals (Singh and Srivastava 2010). In the present study, fish exposure to BPA caused significant decreases in erythrocytes (RBC) and significant increases in MCV, MCH and MCHC parameters in fishes exposed to BPA compared to the control fish. In agreement with our results, Elvin *et al.* (1793) reported that BPA exposure caused a decrease in the RBC fish exposed to BPA, with a significant reduction in the highest sub-lethal concentration (0.4 ppm).

Aiswarya and James (2016) opined that BPA exposure caused a decrease in the RBC count and an increase in the WBC count in Heteropneustes fossilis exposed to BPA. Tilapia treated with 0.52 mg/l pendimethalin showed a significant decrease in the WBC and RBC count, Hb concentration and haematocrit level compared to the control group, Hamed and El-Sayed (2019). Krishnapriya *et al.* (2017) reported significant decreases in haemoglobin, haematocrit, MCV and MCH values, a significant increase in WBC and biphasic trend in RBC count and MCHC values in Labeo rohita fish exposed to sublethal concentration of BPA for 7, 14, 21, 28 and 35 days. The most important response in A. latus fish exposed to BPA was the reduction of RBC, Ht, Hb, MCV, MCH and MCHC (Yaghoobi *et al.* 2017). Karmakar *et al.* (2021) reported significantly decreased haemoglobin content and total erythrocyte count and significantly increased total leucocyte count in NP exposed fishes, Labeo rohita.

The decrease in the RBC might be probably due to the capacity of BPA to induce oxidative damage to bone marrow cells, suppressing erythropoiesis (Tiwari and Vanage 2017). Elvin *et al.* (1793) observed a marked reduction in haemoglobin concentration in BPA treated fish compared to the control fish. Similarly, a decrease in haemoglobin concentration was observed in fishes exposed to 5 and 6 mg/L of BPA, but this decrease was statistically insignificant in this study. The significant decrease in derived haematological indices, MCV, and MCH imply further the possible shrinkage of RBCs as a result of BPA induced toxic stress

Exposure to BPA was reported to increase total leucocyte count in fishes (Aiswarya and James 2016; Krishnapriya *et al.* 2017). Sugita-Konishi *et al.* (2003) study reports contradictory results, showing a significant reduction in the WBCs of BPA exposed fish, which is indicative of a suppressed immune system. In our study, we found significantly increased total leucocyte count in fishes exposed to low doses of BPA (4 mg/L), whereas the decrease in total leucocyte count was observed in fishes exposed to high doses of BPA (5 and 6 mg/l), but this decrease was insignificant from control.

Gene expression

Cytochrome P450 aromatase is an enzyme that catalyzes the conversion of androgen to estrogen and plays an important physiological function in various tissues by regulating estrogen biosynthesis. Expression of

aromatase genes in vertebrates has been observed in a variety of cells and tissues, including the ovary and testis (Silberzahn *et al*. 1988). Kitano *et al.* (1999) reported high expression of P450 aromatase mRNA in the ovary and spleen but weak expression in the testis and brain. Aromatase activity is low in differentiating testes but increases in differentiating ovaries in European pond turtle embryos (Emys orbicularis) (Desvages and Pieau, 1992). In Tilapia, P450 arom-like immunoreactivity was reported in differentiating ovaries but not in differentiating testes (Chang *et al*. 1997).

In this study, ovarian aromatase CYP 450 gene expression is significantly higher in gonads of BPA exposed fishes. Similar to our study, Sudhakumari *et al.* (2005) demonstrated changes in the ontogenic expression patterns of cytochrome OP450 aromatases in whole gonads. The increased P450 arom gene observed in the present study correlates with the increased plasma estrogen levels. Similar to our results, Desvages *et al.* (1993) reported increased P450arom activity and estrogen content in the gonads of embryos incubated at a feminizing temperature, decreased P450arom activity and estrogen content in the gonads of embryos incubated at a masculinizing temperature in the marine turtle (Dermochelys coriacea).

The high temperature was reported to suppress the expression of the P450 arom gene in both female and male larvae Paralichthys olivaceus Kitano *et al.* (1999). Aromatase activity in the gonads of genetic females (ZW) and genetic males (ZZ) during sex differentiation in urodele amphibia (Pleurodeleswaltl) larvae was reported to be higher than in the gonads of genetic males (ZZ). The gonads of sex-reversed males (ZW) created by exposing larvae to high temperatures showed minimal aromatase activity, just like genetic males (Chardard *et al.* 1995).

p53 is a 53-kDa nucleo phosphoprotein and plays a pivotal regulatory role in cell cycle checkpoints, genetic stability, apoptosis and DNA repair. Abu-Qare and Abou-Donia (2001) suggested that alteration or overexpression of the p53 gene may be a biomarker of apoptosis induced by environmental chemicals. In the present study, p53 expression was increased in fishes treated with Bisophenol but reduced in the high dose group (6 mg/L). Lee *et al.* (2008) reported that Kryptolebias marmoratus juveniles exposed to BPA caused initial up-regulation at an early phase of (3-6 h) and then down-regulation. p53 gene would be involved in cellular defence mechanism in the early stage of exposure to endocrine disruptors and long-term exposure may suppress its expression.

Ibrahem *et al.* (2013) reported decreased expression level of p53 gene in O. niloticus treated with dried Spirulina platensis (SP) treated groups in a time-dependent manner. Min *et al.* (2003) studied the expression p53 gene in the liver of Oryzias latipes exposed to BPA for 10 days and reported that the expression of p53 was increased at the maximum level with 2 days of exposure and then gradually decreased for the remaining period of experiment. Soares *et al.* (2012) reported a significant modulation of transcription of p53 gene expression in zebrafish (Danio rerio) exposed to chronic low levels of ethinylestradiol (EE2).

In the present study, expression of gonadal GST was significantly increased in O. niloticus exposed to BPA and the increase was concentration-dependent. Similar to our results, Kim *et al.* (2010) reported high GST gene expression in the liver of pufferfish Takifugu obscures treated with cadmium compared to the other tissue. Richter *et al.* (2011) reported up-regulation of GST expression in the brain of female zebrafish (Danio rerio) exposed to methyl mercury. Glutathione S-transferases

(GST) represent a supergene family of phase II enzymes that provide cellular protection against the toxic effects of various environmental chemicals. It is involved in defence against oxidative stress and electrophilic chemicals. Induction of GST expression suggests that the detoxification system in the ovary was activated due to exposure to BPA and it may be an oxidative stress-related adaptive measure in response to the drug and its metabolites (Alkaladi *et al.* 2020).

Conclusion

The results of the present study revealed that Bisphenol A exposure to 96 h caused mortality in O. niloticus and also induced changes in behavioural patterns. Changes in haematological and biochemical parameters observed in O. niloticus exposed to sublethal concentration of Bisphenol for 7 days were decreased RBC count, increased MCH, MCV and MCHC. These changes in haematological indicators can be utilized to track the negative effects of BPA in the aquatic environment and assess the physiological health of fish. Histological alterations in the kidney, gill gonad and brain tissue were also observed in BPA exposed fishes. BPA treated fish gonad gene increased gonadal OP450 expression in BPA exposed could affect sexual development and differentiation and increased gonad P53 gene expression may lead to unregulated cell division and DNA repair. The increase in the expression level of GST gene in BPA exposed is an adaptive mechanism of fishes to detoxify BPA induced oxidative stress and GST could serve as a potential biomarker. The present study indicates bisphenol A exposure in fish should be viewed as a severe threat to the aquatic ecology as well as endemic and cultivated fish species like Tilapia. Further investigations are required to study the impact of BPA on other aquatic species and the molecular mechanism of its toxicity.

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Author Contributions Statement

S.L.T performed the work as part of his PG research work; S.P.S. provided overall guidance; U.A. conceptualized and designed the experiment; H.N. analyzed the data and drafted the manuscript; S.S.A. Supervised the findings of this work.

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The authors declared no conflict of interest.

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Table I. Primers used for qRT-PCR to relative gene expression analysis

S.no.	Target gene	Primer Code	Reference
1	P53 FP	GCATGTGGCTGATGTTGTTC	Ibrahem *et al.*, (2013)
	P53 RP	GCAGGATGGTGGTCATCTCT	
2	oP450arom FP	5'GCTGAAGAACGGAAACTATACTTCAC 3'	Sudhakumari *et al.* (2005)
	oP450arom RP	5'TGAAGCTGTCTCTCACCCACAACAGC 3'	
3	GST FP	ATG TCA AGA CTG AAG CTA TAC TTT G	AK (2019)
	GST RP	TTA AAT CTT GGA TGC CAG GAA GTG	
4	B- Actine FP	5' GGCATCACACCTTCTACAACGA 3'	
	B- Actine RP	5' ACGCTCTGTCAGGATCTTCA 3'	Yoshiura *et al.* (2003)

Table II. Mortality of fish (O. niloticus) exposed to different concentrations of Bisphenol A

Group	Dose (mg/l)	Cumulative no. of fish dead				Total no. of fish Survived	Survival rate (%)
		24 h	48 h	72 h	96 h		
C	0	0	0	0	0	10	
T1	7	0	1	4	6	4	40.00
T2	9	1	3	5	7	3	30.00
T3	11	2	4	6	8	2	20.00
T4	13	3	4	6	8	2	20.00
T5	15	3	5	7	10	0	0.00
T6	17	9	9	10	10	0	0.00
T7	19	9	10	10	10	0	0.00

Table III. Median lethal concentration (LC50) of Bisphenol A on O. niloticus up to different times of exposure

Time (in h)	LC50 (mg/L)	Confidence interval (95%)
24	14.307	12.888 – 16.096
48	12.190	10.513 - 14.00
72	9.109	6.250 – 10.861
96	6.794	3.127 – 8.501

Table IV. Effect of haematological parameters (Mean ± SD) in fishes exposed to BPA

Parameters	Control	T1 (4mg/L)	T2 (5mg/L)	T3 (6mg/L)
Hb (gm/dl)	6.19 ± 0.12	6.04 ± 1.15	4.58 ± 2.78	4.27 ± 1.47
Leukocyte (cells/cumm)	110.69^{a*} ± 0.45	147.08^{b*} ± 23.25	104.51^{a*} ± 10.34	95.12^{a*} ± 28.46
Erythrocyte (Million/cumm)	3.26^{b*} ± 0.15	1.45^{a*} ± 0.39	1.06^{a*} ± 0.903	1.49^{a*} ± 1.34
Haematocrit (%)	17.24 ± 0.15	17.08 ± 5.09	12.17 ± 8.86	13.34 ± 5.42
MCV (FI)	53.71^{a*} ± 0.06	116.01^{c*} ±3.12	85.22^{b*} ±31.92	100.47^{bc*} ± 17.4
MCH (Picograms)	19.27^{a*} ± 0.15	40.94^{bc*} ± 18.71	28.98^{ab*} ± 20.77	48.11^{c*} ± 21.91
MCHC (g/dl)	36.05^{a*} ± 0.01	43.18^{ab*}± 13.63	31.95^{a*} ± 20.57	54.13^{b*} ± 15.63

*Significant at p<0.05; Means in same row bearing different superscript differ significantly.

Table V. Expression of gonadal gene in BPA exposed fish (Mean ± SD).

Gene	T0	T1 (4 mg/l)	T2 (5 mg/l)	T3 (6 mg/l)
GST	1.09^{d*} ± 0.59	3.44^{c*} ± 0.17	4.70^{b*} ± 0.50	9.17^{a*} ± 0.46
OP 450	1.46^{d*} ± 1.07	5.31^{b*} ± 0.25	7.55^{a*} ± 0.40	3.49^{c*} ± 0.33
P53	1.33^{c*} ± 0.93	7.52^{ab*}± 0.36	9.32^{a*}± 0.90	5.64^{b*} ± 0.54

*Significant at p<0.05; Means in same row bearing different superscript differ significantly.

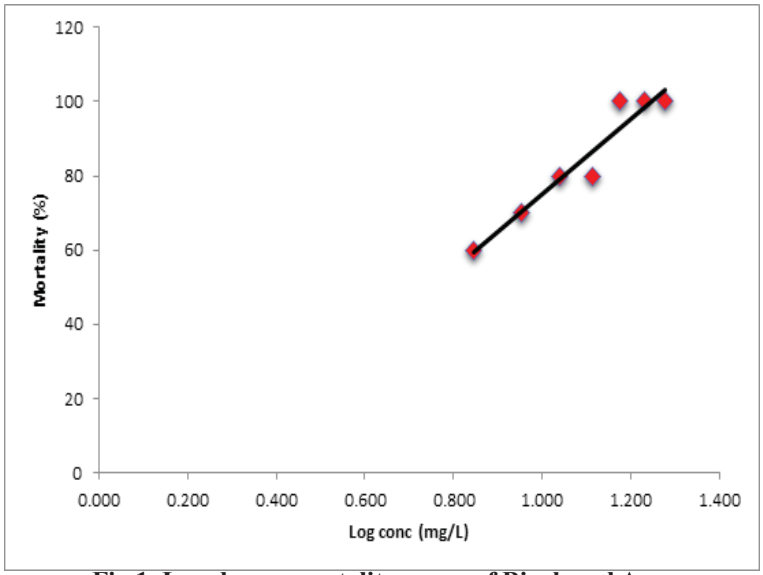
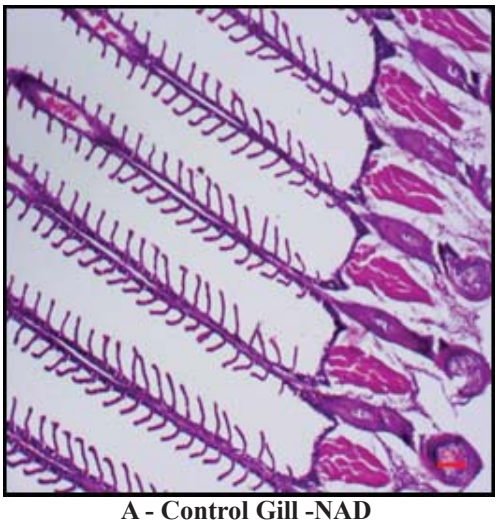


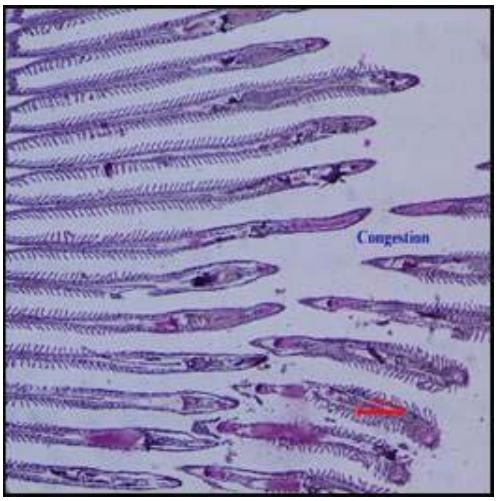
Fig.1. Log dose – mortality curve of Bisphenol A on *Oreochromis niloticus* in 96 h



A - Control Gill -NAD

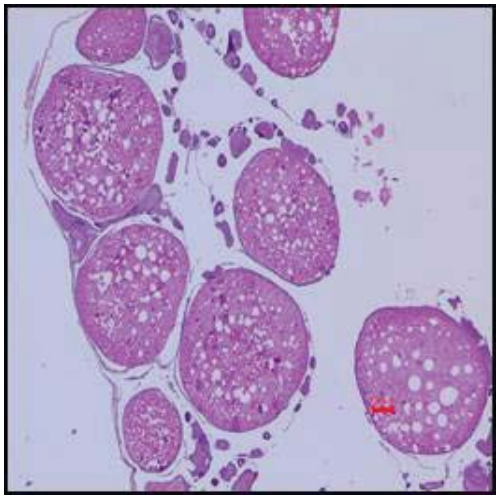
B – T1 – Mild congestion & fusion of villi

C- T2 – Mild Congestion & fusion of villi

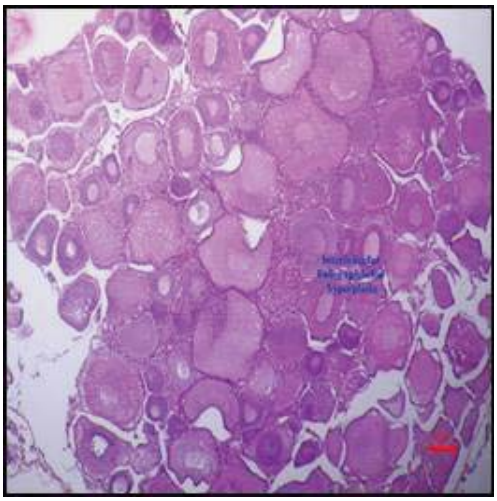


D- T3 – Congestion of villi

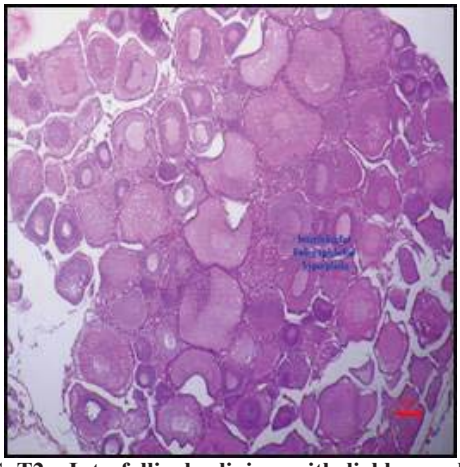
Fig 2. Histopathology of Gills



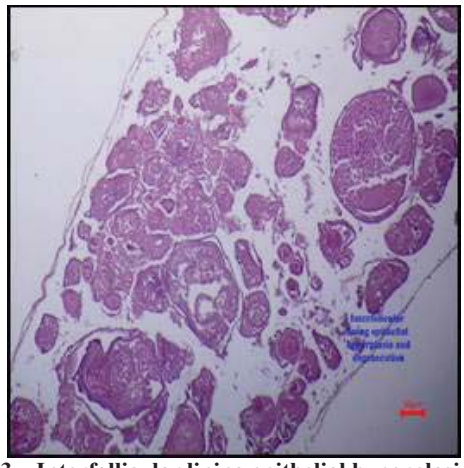
A - Control Gonad -NAD



B – T1 – Interfollicular lining epithelial hyperplasia

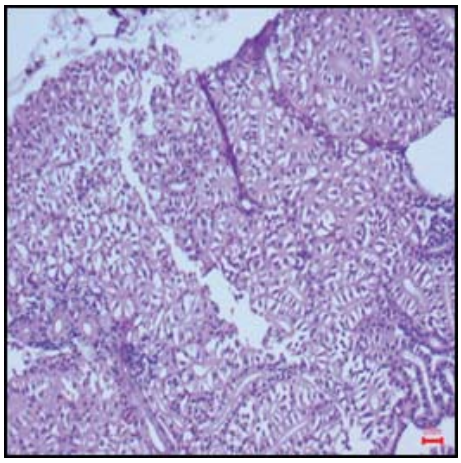


C- T2 – Interfollicular lining epithelial hyperplasia

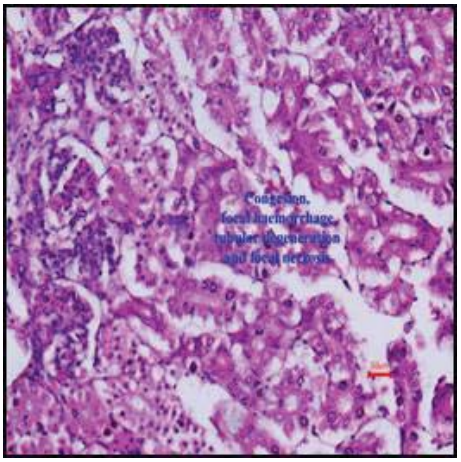


D- T3 – Interfollicular lining epithelial hyperplasia and degeneration

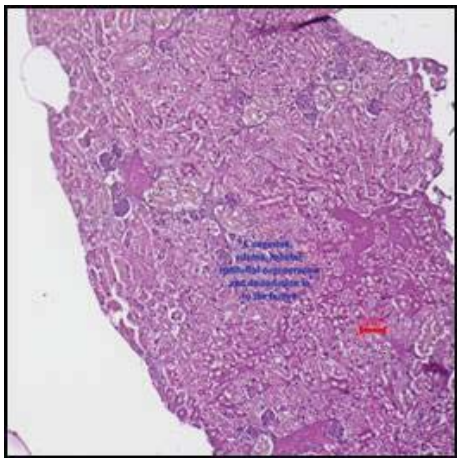
Fig 3. Histopathology of Gonad



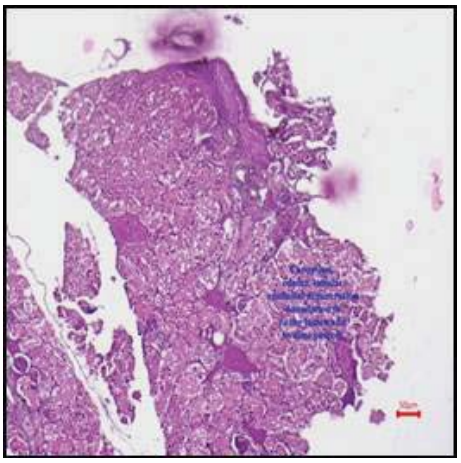
A - Control -NAD



B – T1 – Congestion, focal degeneration and focal necrosis

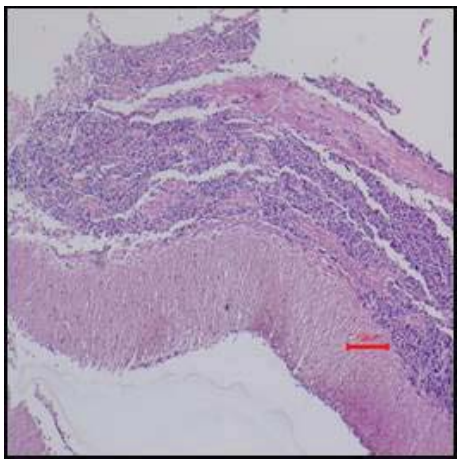


C- T2 – Congestion, edema, tubular epithelial degeneration and denudation in to the lumen

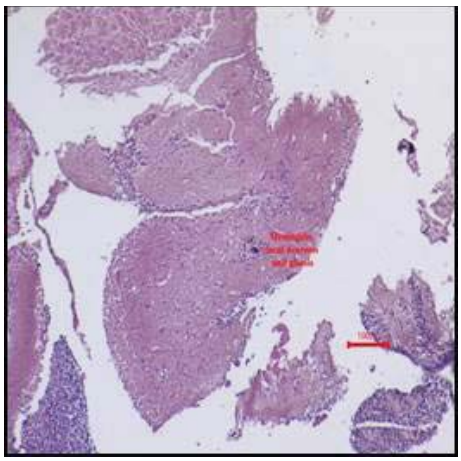


D- T3 – Congestion, epithelial tubular degeneration, denudation in to the lumen and hyaline casts

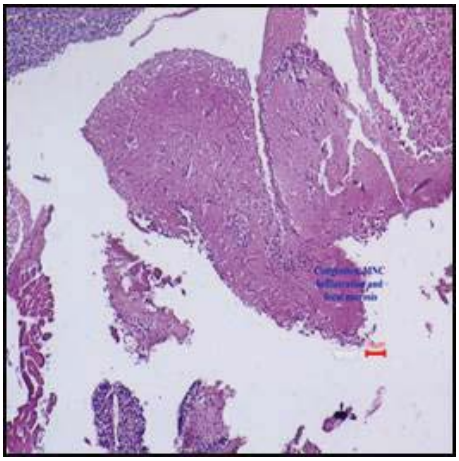
Fig 4. Histopathology of Kidney



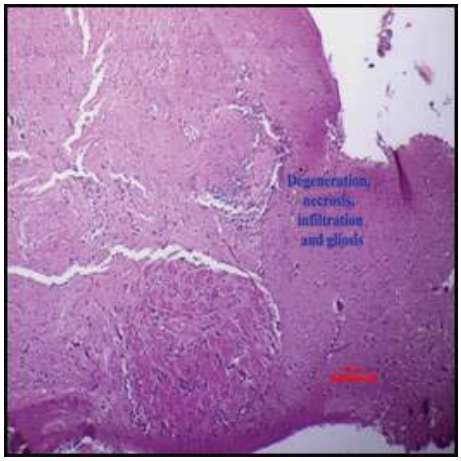
A - Control -NAD



B – T1 – Meningitis, focal necrosis and gliosis



C- T2 – Congestion, mononuclear cell infiltration and focal necrosis



D- T3 – Degeneration, necrosis, infiltration and gliosis

Fig. 5. Histopathology of Brain

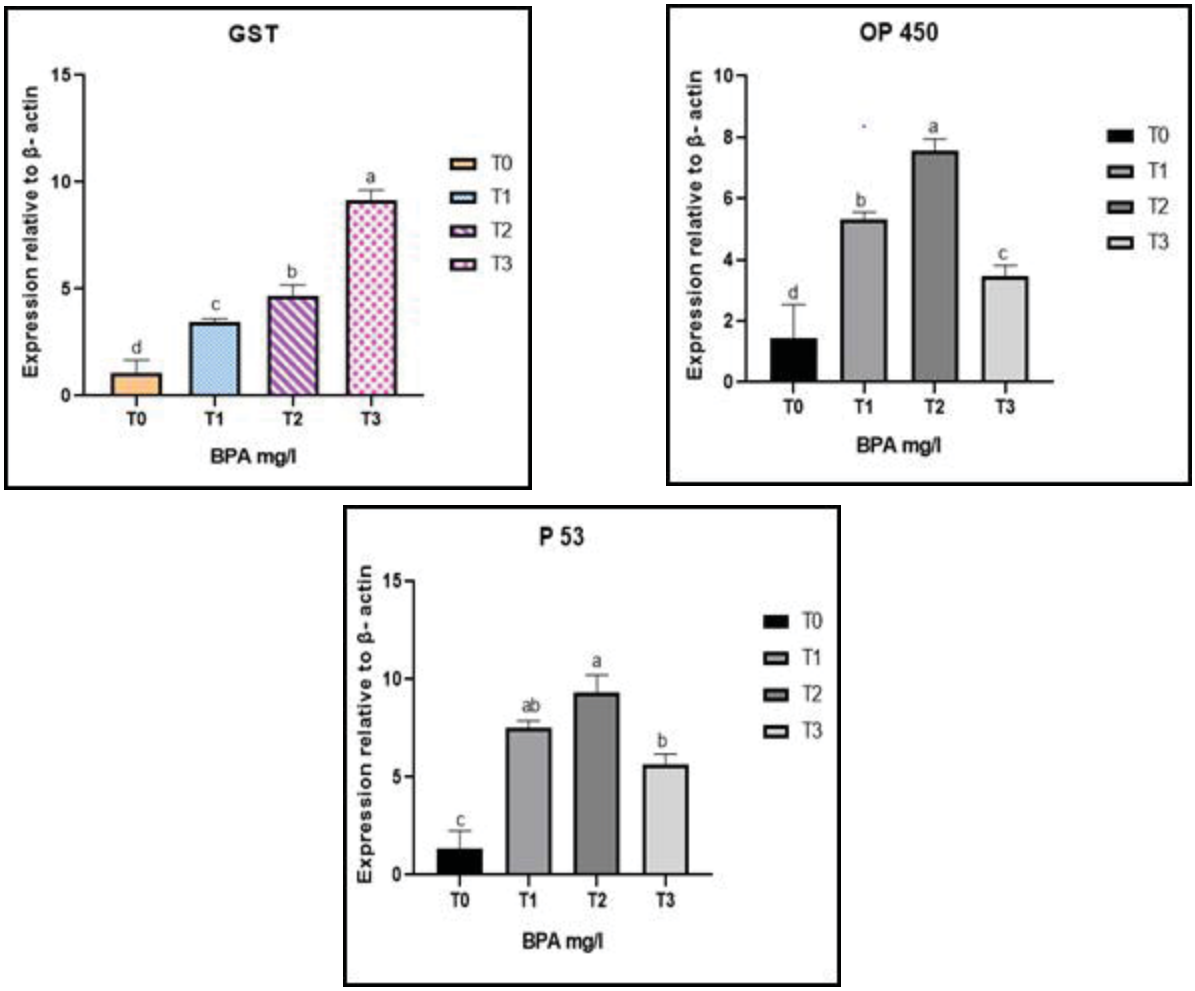


Fig. 6. Effect of BPA treatment on gonadal gene (GST, oP450 and P53) -expression in *O. niloticus*