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Research Paper

Histopathological and Biochemical Responses to Sublethal Concentrations of Bisphenol A in Female *Heteropneustes fossilis*

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Abstract: Bisphenol A (BPA) is a significant global industrial pollutant frequently detected in surface waters, residues, and biota. This study investigates the histopathological and biochemical effects of BPA on female *Heteropneustes fossilis* exposed to various sublethal concentrations. The median lethal concentration (LC50) of BPA for *Heteropneustes fossilis* was determined to be 7.1443 mg/L over a 96-hour period using probit analysis. Fish were subjected to sublethal concentrations of 0.714 mg/L (1/10th), 1.428 mg/L (1/20th), and 2.142 mg/L (1/30th) (Groups II, III, and IV) with ten specimens per aquarium for 28 days. Post-exposure, fish were dissected, and ovarian tissues were collected for histopathological examination. Blood samples were obtained by severing the caudal peduncle and analyzed for total plasma protein content, glucose, Aspartate aminotransferase (AST/GOT, EC 2.6.1.1), Alanine transaminase (ALT/GPT, EC 2.6.1.2), and 17β-Estradiol (E2).
Results indicated that BPA exhibits considerable toxicity to *Heteropneustes fossilis*, with effects being concentration-dependent. Sublethal BPA exposure led to significant structural alterations in the ovaries, including necrosis, increased oocyte atresia, perifollicular cell hyperplasia/hypertrophy, reduced vitellogenesis, and changes in gonadal staging. Additionally, BPA exposure resulted in a marked decrease in plasma protein levels and a significant, progressive increase in AST, ALT, and plasma E2 levels across all experimental groups. These findings suggest that sublethal concentrations of BPA can disrupt endocrine function and reproductive health in adult fish, warranting further research into its impacts.

Keywords: Bisphenol A, *Heteropneustes fossilis*, histopathology, biochemistry, endocrine disruption, reproductive toxicity

Introduction:

Bisphenol A (BPA) has emerged as a critical environmental contaminant and endocrine disruptor, posing significant

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ecological and health concerns. Aquatic environments have become substantial repositories for BPA, contributing to widespread contamination. Prior research has consistently highlighted the detrimental effects of anthropogenic toxicants, including BPA, on fish organs (Faheem et al., 2016; Reddy, 2017; Ratn, et al., 2018). Currently, global BPA production reaches approximately 3 billion kilograms annually, with over 100 tons released into the atmosphere each year (Vandenberg et al., 2009). Inefficiencies in wastewater treatment processes result in BPA persistence in effluents, leading to contamination of aquatic ecosystems (Staples et al., 1998; Kang et al., 2006).

BPA functions as an anti-androgen, anti-estrogen, and steroidogenic enzyme inhibitor, interfering with steroid hormone action and production, thereby disrupting reproductive fitness. The U.S. Environmental Protection Agency (USEPA) reports that approximately one million pounds of BPA are discharged into the environment annually, often detected in municipal wastewater (Erler & Novak, 2010). Laboratory studies have underscored BPA's potential as an endocrine disruptor, closely mimicking estradiol's structure and function by binding to and activating estrogen receptors (Takayanagi et al., 2006; Takahashi et al., 2018). BPA's environmental persistence and bioaccumulation in tissues amplify these concerns.

The effects of endocrine-disrupting chemicals (EDCs) such as BPA differ from traditional pollutants, with impacts that may not be immediately apparent. Exposure to EDCs during developmental stages can induce permanent structural changes, manifesting later in life (Frye et al., 2012). This research aims to elucidate whether BPA can disrupt fish reproduction through alterations in endocrine function. In fish, monitoring ovarian growth, structure, and sex steroid hormone concentrations provides valuable insights into the effects of EDCs in wild populations (Hecker et al., 2002). Consequently, this thesis investigates the histopathological changes in the ovaries and variations in serum estrogen (17β-estradiol) hormone concentrations in the catfish *Heteropneustes fossilis* following exposure to BPA. The study aims to advance understanding of BPA's impact on endocrine and reproductive health in aquatic organisms.

Materials and Methods:

Healthy adult *Heteropneustes fossilis* were selected during the spawning season of 2016 from local aquaculture ponds. These fish were acclimatized to laboratory conditions for 15 days prior to experimentation. Bisphenol A (BPA) [2,2-Bis (4-hydroxyphenyl)propane, CAS Number: 80-05-7, 97% purity] was sourced from Shreeji Pharma International, Vadodara, Gujarat, India. Groups of 10 healthy fish (average weight: 36.78 g) were exposed to various concentrations of BPA to determine the median lethal concentration (LC50) using the probit analysis method (Finney, 1952). The LC50 of BPA for *H. fossilis* was calculated to be 7.142 mg/L.

For the main experiment, fish were exposed to sublethal BPA concentrations of 0.714 mg/L (1/10th LC50), 1.428 mg/L (1/20th LC50), and 2.142 mg/L (1/30th LC50) (designated as Groups II, III, and IV, respectively). Each group comprised ten fish housed in separate aquaria for 28 days. At the conclusion of the exposure period, the fish were euthanized and dissected to obtain ovarian tissue for histopathological examination. Blood samples were collected via caudal vein puncture using ethylene diamine tetraacetic acid (EDTA) as an anticoagulant.

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Histopathology:

Ovarian tissues were fixed and processed through a graded ethanol series, cleared in xylene, and embedded in paraffin wax with a melting point of 60°C. The paraffin blocks were sectioned at 6 µm using a rotary microtome, mounted on glass slides, and stained with hematoxylin and eosin (H&E). Sections were examined under a light microscope (NIKON ECLIPSE E 400, USA) and photographed using a digital camera attached to the microscope.

Biochemical Studies:

Total Protein Content: Estimated using a modified version of the standard method by Lowry et al. (1951).

Blood Glucose: Determined using the Folin-Malmros micro procedure as modified by Murrell and Nace (1958).

Aspartate Aminotransferase (AST/SGOT, EC 2.6.1.1): Assayed following the method of Friedemann et al. (1943).

Alanine Aminotransferase (ALT/SGPT, EC 2.6.1.2): Assayed according to the method prescribed by Mohun and Cook (1957).

Hormone Assay: Serum estrogen (17β-estradiol) levels were measured using a specific radioimmunoassay (RIA) as described by Jyotsna and Medhamurthy (2009).

Results:

Ovarian Histopathology

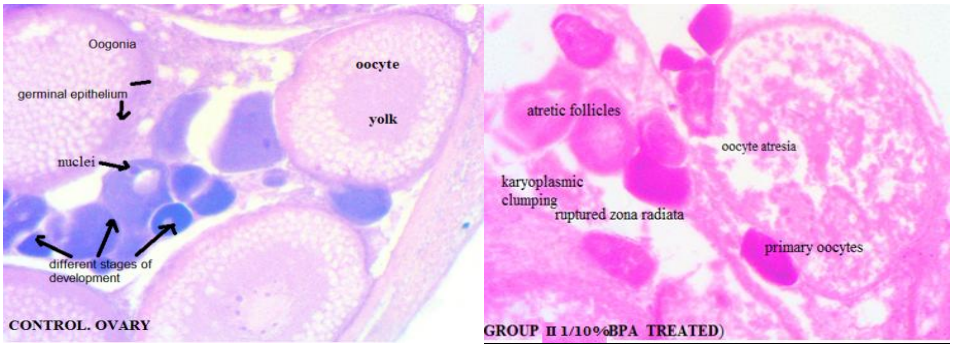
Histopathological analysis was conducted to assess ovarian damage in fish exposed to sublethal concentrations of Bisphenol A (BPA). At the start of the experiment, both control and treated groups displayed similar ovarian stages. However, significant deformities were observed in the ovarian sections of BPA-exposed fish compared to the control group.

Group II (1/10th LC50 BPA): Fish exhibited increased oocyte atresia, perifollicular cell hyperplasia/hypertrophy, and decreased vitellogenesis. Additional observations included gonadal staging vacuolation, necrosis, degeneration, and ruptured ovarian walls with reduced lumen.

Group III (1/20th LC50 BPA): The ovarian tissue showed atretic follicles, karyoplasmic clumping, necrosis, and ruptured zona radiata.

Group IV (1/30th LC50 BPA): More severe structural anomalies were observed, including marked degeneration of oocytes, atretic follicles, and significant damage to the zona radiata.

In all experimental groups, oocytes displayed irregular shapes and disintegrated cytoplasm, in contrast to the control fish, which maintained normal oocyte morphology (Plate I). These findings indicate that even at sublethal concentrations, BPA exposure results in substantial histopathological alterations in the ovaries of *Heteropneustes fossilis*.



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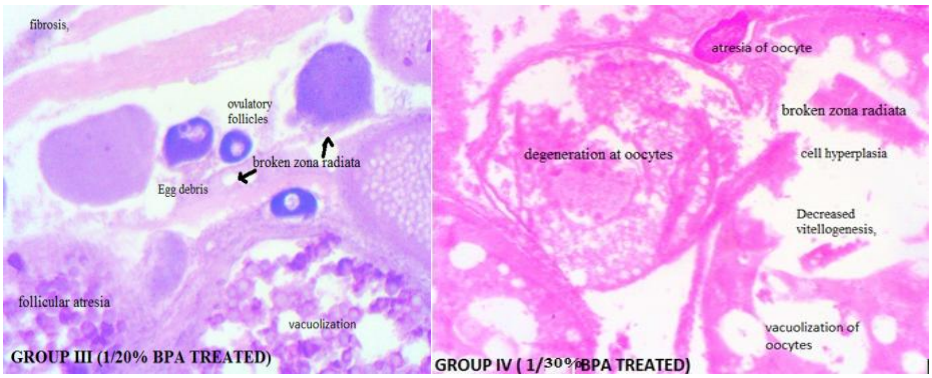

Plate I. FIG.1-4. Plate I: Histopathological effects of sublethal BPA concentrations on the ovaries of *Heteropneustes fossilis* are illustrated in Fig. 1-4. **(A)** The control ovary shows normal structures with oogonia and developing oocytes. **(B)** Group II (1/10th LC50 BPA) displays atretic oocytes, ruptured zona radiata, karyoplasmic clumping, necrosis, and nuclear hypertrophy. **(C)** Group III (1/20th LC50 BPA) reveals cellular degeneration, follicular atresia, egg debris, broken zona radiata, and fibrosis. **(D)** Group IV (1/30th LC50 BPA) demonstrates severe degeneration, atrophy, germ cell syncytia, hypertrophy, pyknosis, vacuolated oocytes, and reduced vitellogenesis (H&E staining, x400 magnification).

Table. 1. Changes in the Biochemical and hormonal parameters in *H. fossilis* treated with sub lethal concentration of bisphenol A.

Parameter	Control (Group I)	Group II	Group III	Group III
Plasma protein(µg/ml)	9.66±0.96	8.37±0.27	6.56±0.67	4.58±0.67
Plasma Glucose (mg/dl)	98.3±2.15	106.1±2.4	124.5±3.63	143.5±4.5
AST (SGOT) (U/L)	60.8± 0.74	71.28± 1.2	88.94± 2.4	124.22± 3.8
ALT (SGPT) (U/L)	29.7± 0.33	34.8± 0.46	41.2 ± 0.48	49.6± 0.77
17-estradiol (E2) (ng/ml	2.4±0.45	2.7±0.55	4.9±0.6	6.1±0.82

Biochemical Studies:

The results of biochemical analyses are summarized in Table 1. Exposure to various concentrations of BPA resulted in a notable decrease in plasma protein levels across all experimental groups. Additionally, glucose levels were elevated in all treated groups (Table 1). Furthermore, both AST (SGOT) and ALT (SGPT) levels (Fig. 5 and Table 1) exhibited a consistent and significant increase in all BPA-exposed fish. Moreover, 17β-estradiol (E2) levels showed a gradual and significant rise in all experimental groups treated with BPA.

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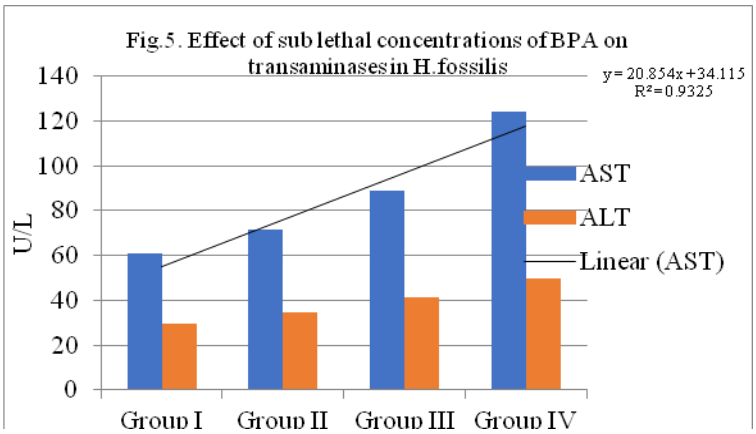

Discussion:

The developmental stage of gonads is crucial for assessing reproductive health in organisms exposed to environmental stressors, including natural and synthetic chemicals such as endocrine disrupting compounds (EDCs) (Muthulakshmi et al., 2018; Haq and Raj, 2019). Our study focused on *Heteropneustes fossilis* to understand how Bisphenol A (BPA) affects ovarian development and hormone levels, crucial for reproductive function. Initially, all experimental groups exhibited similar ovarian stages, but exposure to sublethal BPA concentrations induced dose-dependent structural changes. These changes included disintegrated oocyte cytoplasm, cellular degeneration, nuclear hypertrophy, follicular atresia, and fibrosis, as observed microscopically. While previous studies have highlighted BPA's reproductive toxicity, detailed mechanisms impacting the hypothalamus-pituitary-gonadal (HPG) axis and steroidogenesis in *H. fossilis* remain unclear (Dutta, 2017; Milton et al., 2017; Karnatak et al., 2018; Lal, 2018). Our findings underscore BPA's ability to disrupt ovarian histology, potentially reducing fecundity and altering offspring quality, indicating impaired enzyme systems, hormonal secretion, and ovarian regulation (Kumar and Ali, 2014). Further investigations are needed to elucidate the genetic pathways underlying these histopathological changes (Faheem et al., 2017).

Biochemical Studies:

Aminotransferases (ALT and AST) are sensitive indicators of liver damage (Reddy, 2012). Elevated ALT and AST levels in all BPA-treated groups suggest liver cell damage, corroborated by histopathological observations. Increased enzyme activity may result from cellular permeability changes and hepatocyte damage, disrupting metabolic pathways and impacting various tissues under BPA stress (Ajaz Ahmad Rather, 2015; Kavya et al., 2016; Mishra et al., 2016; Dar et al., 2018). Additionally, decreased plasma protein levels in BPA-exposed groups indicate impaired protein synthesis or renal excretion, potentially exacerbated by oxidative stress (Banerjee et al., 2017). Elevated glucose levels reflect increased energy demand due to BPA-induced stress, impacting glycolytic pathways and metabolic homeostasis in fish (Madhusudan et al., 2003; Osman et al., 2010). These biochemical alterations highlight BPA's systemic effects beyond reproductive disruption.

Effects of BPA on Sex Hormones:

BPA acts as an endocrine disruptor, interacting with estrogen receptors (ERα and ERβ), altering steroid biosynthesis pathways (Takayanagi et al., 2006). Our

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study observed elevated 17β-estradiol (E2) levels in BPA-exposed fish, suggesting enhanced aromatase enzyme activity converting testosterone to E2 (Simpson et al., 1994). This disruption of estrogen balance may impair reproductive functions regulated by the hypothalamus-pituitary axis, affecting gonadal maturation and hormone feedback mechanisms (Itskovitz et al., 1991; Shaw et al., 2010). The observed hormonal changes in *H. fossilis* align with studies on other aquatic vertebrates, indicating BPA's potency as an estrogenic disruptor (Kime et al., 1999; Whitehead and Rice, 2006). Future research should explore species-specific responses and long-term implications of BPA exposure on fish reproductive health.

Conclusion:

BPA poses significant public health concerns due to widespread exposure through food and water. Despite debates on low-dose effects, evidence suggests BPA may disrupt reproductive functions observed in *H. fossilis*. As a resilient species, *H. fossilis* exhibited varied behavioral, biochemical, and reproductive anomalies when exposed to sublethal BPA concentrations. Continued research is essential to fully understand the scope of BPA's impact on aquatic ecosystems and human health, emphasizing the need for stringent environmental regulations and monitoring programs.

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