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4-TERT-BUTYLPHENOL-INDUCED REPRODUCTIVE ENDOCRINE DISRUPTION IN THE FRESHWATER STINGING CAT FISH *HETEROPNEUSTES FOSSILIS*

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Abstract: To assess the impact of 4-tert-Butylphenol (4-tBP), an endocrine-disrupting chemical (EDC), on the reproductive cycle of the fish model, *Heteropneustes fossilis,* biochemical assays and gene expression studies were employed. The median lethal concentration (LC_{50}) of 4-tBP was estimated before subjecting the fishes to antioxidant enzyme assay (catalase: CAT; superoxide dismutase: SOD) and gene (*cyp19a1a*: ovarian aromatase; *vtg*: liver vitellogenin) expression studies. After following a standardized set of protocols, the LC_{50} (96 hours) value for the exposure of the compound in fish was found to be 27.8 mg/L. The SOD and CAT antioxidant enzyme activity, at one-tenth of the LC_{50} (96 hours) value of 4-tBP, was found to be disrupted; thereby, suggesting an increase in the oxidative stress in the fish. Lastly, the same fish tissue samples were taken for gene expression studies, which revealed the upregulation and downregulation of *vtg* and *cyp19a1a* genes, respectively. The upregulation of the *vtg* gene expression and the enhanced synthesis of associated (*vtg*) protein can be attributed to the structural similarity of 4-tBP with that of the natural estrogens to bind to the receptor, which leads to the downstream protein synthesis mechanism. In contrast, the downregulated *cyp19a1a* gene can be associated with impairments in ovarian development that can alter the reproductive physiology. Therefore, these results suggest the endocrine-disrupting nature of the compound 4-t-BP in *H. fossilis.*

Keywords: Aromatase, EDCs, *Heteropneustes fossilis,* Tert-Butylphenol, Vitellogenin.

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INTRODUCTION

Alkylphenols, termed EDCs, are environmental contaminants sourced from natural or anthropogenic activities (Tripathi and Chaube, 2024). These contaminants pollute the water bodies heavily, leading to lipophilic alkylphenols (APs) accumulation and toxicity in aquatic animals (Selvaraj *et al.,* 2014). The APs are reported to alter the normal endocrine functions in the bodies of fish, humans, and other animal species by interfering with the effects of endogenous sex hormones, thereby adversely

affecting the reproduction and developmental processes (Gautam and Chaube, 2021). The need for research on the effects of EDCs (such as 4-tBP) becomes more significant, as these chemicals can lead to the loss ofbiodiversity in thelong term.

The compound 4-tert-Butylphenol (Fig. 1a and 1b) is an endocrine-disrupting chemical (with its hazard classification, as shown in Fig. 1c) that is derived from phenol and is characterized by the presence of a tert-butyl group on its aromatic ring (Hoang and Park, 2024). Recent research studies have suggested the hepato-toxic nature of this compound along with disrupting the metabolic pathways in fishes, such as zebrafish (Wang *et al.,* 2024), common carp (Cui *et al.,* 2025) and others. Apart from the liver toxicity, the 4-tBP has also been reported to exhibit an endocrine-disrupting property in male *Cyprinus carpio* as the fish reportedly produced vitellogenin, a female-specific egg yolk protein, when exposed to 4-tBP (structurally and functionally similar to many natural estrogenic hormones); thereby, making vitellogenin as a key biomarker for EDC exposure in fish ecotoxicological studies (Kime *et al.,* 1999; Barse *et al.,* 2006). Despite several research studies having been conducted in the domain of EDC toxicity, the effect and underlying mechanism of 4-tBP toxicity in the hypothalamic-pituitary-gonadal (HPG) axis of the fish model, *Heteropneustes fossilis* (Bloch, 1794), is still unreported.

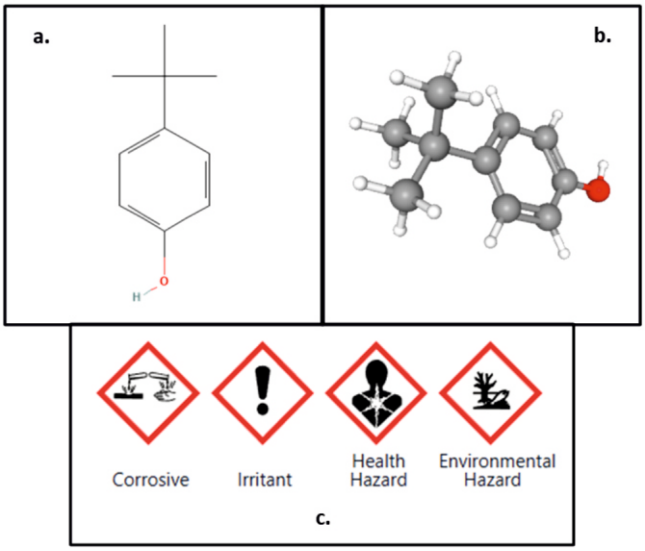

Fig. 1: The structure: (a) 2D and (b) 3D; and GHS (Globally Harmonized System) pictograms (c) of 4-tert-Butylphenol (PubChem [accessed on 14/12/2024]).

Heteropneustes fossilis (Bloch, 1794), commonly known as the stinging catfish, is a freshwater fish speciesnative to South and Southeast Asia, including countries like India, Bangladesh, and Sri Lanka (Ratmuangkhwang *et al.*, 2014; Verma, 2017). This fish (as shown in Fig. 2, along with its key descriptors in Table 1) is well-adapted to diverse aquatic environments such as ponds, swamps, and ditches andcan even tolerate slightly brackish water (Verma and Prakash, 2016; Loganathan *et al.*, 2024). This fish species is a vital food source and holds cultural and medicinal significance in the regions where it is found (Verma, 2016; Prakash and Verma, 2016; Nasreen and Borah, 2023; Shukla and Prakash, 2024).

Owing to the multiple significances of *H. fossilis*, it becomes crucial to study the effects of EDCs on its HPG axis-associated genes, proteins, and metabolites. This understanding will aid in the conservation ofthis fish species along with human needs. Therefore, this research study is focused on assessing gene (*cyp19a1a, vtg*) expression, along with other biochemical parameters, against the 4-tBP exposure of the fish. Different multidisciplinary approaches, including bioinformatics tools such as molecular docking (Huang *et al.*, 2024; Srivastava *et al.*, 2021), molecular dynamics simulations (Salahshoori *et al.*, 2024) and multi-omics (Fabbri *et al.*, 2023) are required to understand the behaviour of EDCs.

The ovarian aromatase (*cyp19a1a*) gene plays a pivotal role in fish reproduction and sex differentiation. It encodes an enzyme, *cyp19a1a*, responsible for converting androgens into estrogens, which are crucial for ovarian development and maintenance. This gene is predominantly expressed in the ovaries and is essential for female sex differentiation in many fish species. Therefore, any disruption or mutation in the *cyp19a1a* gene can impair ovarian development (Chaube *et al.*, 2015). On the other hand, the liver vitellogenin (*vtg*) gene codes for the *vtg* protein, whose synthesis is induced by the binding of liver estrogenic receptors with estrogen hormones in the blood. These proteins are then released into the

bloodstream and transported to the ovaries, transforming them to enrich the yolk and aid in oocyte maturation and ovarian development (Hara *et al*., 2016). This study was conducted in Varanasi, one of the highly urbanized cities of Uttar Pradesh, India. As discussed earlier, it aimed to understand the toxicological impacts of 4-tBP on the reproductive health of the fish model

Fig. 2: *Heteropneustes fossilis* (Bloch, 1794).

Table 1: Key description of the fish model–*H. fossilis*.

Local name	Singhi
Scientific name	*Heteropneustes fossilis* (Bloch, 1794)
Family	Heteropneustidae
Habitat	Freshwater
Feeding habit	Benthopelagic
Native geography	India, Bangladesh, and Sri Lanka
Conservation status (IUCN)	Least concern (LC)

MATERIALS AND METHODS

Chemicals

The chemicals like 4-tert-Butylphenol (CAS No: 98-54-4; S-3829950/312) and HPLC-grade acetone were procured from Merck, USA; whereas, 50μM EDTA (pH 8), L-Methionine, 0.1M NaOH, Griess reagent (1% Sulphanilamide + 0.1% NED), 10mM Riboflavin, 1%Triton-X, 10mM Hydroxylamine hydrochloride, 0.8% Butyl-hydroxy-toluene, 8% SDS, 20% Acetic acid, 0.8% TBA, Glacial acetic acid, H_2O_2, 5% $K_2Cr_2O_7$ (for enzymes Superoxide dismutase and Catalase), and clove oil (average Eugenol concentration- 80%) were procured from respective local vendors.

Fish collection and acclimatization

The fish (*H. fossilis*) were collected with the help of local fishermen, in their resting phase (December), from different ponds near the Ganga River. A standardized protocol was used to collect the fish (Prakash and Verma, 2015; Kumar *et al*., 2022) while considering their characteristic features- weight: 30- 35g, snout-to-tail length: 14-16cm, age: 1-1.5 yrs. The collected fish were then transported to the laboratory in water-filled containers, acclimatized in the 20 litre tank containing fresh water for two weeks, and feda commercial (fat-free) diet. The external stressors were carefully reduced during the collection of fish samples and their acclimation process. The handling of fish, from their natural habitat to the dissecting tray, was performed under the approved animal care standards, as outlined by the Institutional Animal Ethical Committee (IAEC), BHU, Varanasi.

Determination of median lethal concentration (LC_{50}96hrs)

Determining the LC_{50}96 hrs value is an important parameter that signifies the lethal concentration of a test compound in the air or water to 50% of a test population exposed to it. For this purpose, different glass aquaria were established as test environments to determine the LC_{50}96 hrs value in a controlled experimental setup. These aquaria were filled with dechlorinated water, where varying concentrations of 4-tBP were introduced, as specified in Table 2, ensuring a gradient of exposure levels. The exposure period spanned 96 hours, during which the fish were observed under standardized conditions. To minimize environmental contamination and ensure clean conditions, essential measures were taken into account – the fish were fed daily with their regular fat-free diet, and the water was changed every 24 hours (Barse *et al.,* 2006).

Table 2: 4-tBP exposure concentration for the determination of the LC_{50}96 hrsvalue.

Group	4-tBP Conc. (mg/L)	Number of Fish/tank
1	25.0	10
2	30.0	10
3	35.0	10
4	40.0	10
5	45.0	10

After the 96-hour exposure period, the mortality in each concentration group was recorded, the respective net mortality rates were calculated, and the LC_{50}96 hrs value was determined by Finney's probit analysis method (Ghosh and Saha, 2022).

Exposure treatment of 4-tBP in the fish model

Following acclimatization, the experimental fish were segregated into two groups and housed in separate 10-liter aquaria, designated as the control and treatment groups. The treatment group was subjected to daily exposure to 4-tBP at a concentration of 2.8 mg/L (equivalent to one-tenth of the determined LC_{50}96 hrs value); the control group remained untreated and served as the baseline for comparison. Both groups were fed *ad libitum* using commercially available fish feed (Taiyo, Malaysia), and the water in both the tanks was replaced daily to maintain optimal water quality with reduced contamination. This process included the removal of excess feed and residual 4-tBP from the treatment tank to prevent interference with the experimental conditions (Srivastav *et al.*, 2025).

The duration of exposure was 28 days, during which the fish were monitored under controlled and standardized environmental settings. This experimental setup facilitated a systematic evaluation of 4-tBP's sub-lethal effects on the test subjects while adhering to best practices in aquatic toxicology research.

Tissue sampling

A total of ten fish (from each group) were euthanized promptly using a moderate dose of clove oil to ensure humane treatment. Following euthanasia, the ovary and liver tissues were rapidly excised and collected for downstream analyses.

a) For enzymatic analyses and oxidative stress assays:

The liver tissues were directly stored at -20°C until further processing. This protocol ensured the preservation of tissue quality for both molecular and biochemical analyses (Gautam and Chaube, 2021).

b) For gene expression studies:

The collected ovary and liver tissues were immediately preserved in RNA later and were then stored overnight at 4°C to ensure thorough stabilizationwhile maintaining the RNA integrity. If intended for long-term storage, the sample can also be preserved at -20°C (Chaube *et al.,* 2015).

Sample preparation for the antioxidant enzyme assay

The activities of oxidative stress biomarkers, such as superoxide dismutase (SOD) and catalase (CAT), were measured using a spectro-photometric approach (Gautam and Chaube, 2021). For this purpose, the target tissue was carefully excised and subsequently weighed to determine the required volume of processing buffer. Therefore, a 10% w/v homogenate of the tissue sample was prepared in 1% phosphate-buffered saline (PBS) to provide an optimal environment for enzymatic stability and activity. To remove cellular debris and obtain a clear supernatant for analysis, the prepared tissue homogenates were subjected to centrifugation at a high speed of 12,000xg under refrigerated conditions (4°C) for 30 minutes. The cooling was crucial to maintain the structural and functional integrity of the enzymes during the process. The supernatant containing the homogenate's soluble components, including the enzymes of interest, was carefully collected following centrifugation. This clear solution was used for the subsequent spectrophotometric estimation of enzymatic activities, ensuring precise and reproducible measurements.

a) Determination of superoxide dismutase (SOD) activity:

For the assessment of superoxide dismutase

(SOD) activity, the sample supernatants were incubated with a reaction mixture containing riboflavin and the Griess reagent (Gautam and Chaube, 2021). During incubation, the riboflavin absorbed photons and transitioned to an excited state, interacting with electron donors such as NADH to form reactive riboflavin radicals. These radicals transferred electrons to oxygen molecules, producing superoxide ions, which subsequently reacted with hydroxylamine hydrochloride to produce nitrite ions. These nitrite ions interacted with the Griess reagent– composed of sulphanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride in an acidic medium– through a diazotization reaction, yielding a red azo dye. The absorbance of this dye was measured spectrophotometrically at 540 nm after the incubation period to provide a quantitative estimate of superoxide ion levels. In the presence of SOD, superoxide ions get converted into hydrogen peroxide, leading to a reduction in nitrite formation and, thus, a measurable change in absorbance values, which reflects the enzyme activity.

b) Determination of catalase (CAT) activity:
To determine catalase (CAT) activity, the sample supernatants were incubated with a freshly prepared hydrogen peroxide solution (H_2O_2) as a substrate for the enzyme to exhibit its activity. The reaction was conducted at room temperature to maintain the optimal enzyme activity, whereas the incubation period was standardized for one minute to ensure reproducibility and prevent excessive decomposition of H_2O_2 (Gautam and Chaube, 2021). Following the incubation, the reaction mixture was treated with a coloring reagent that contained potassium dichromate ($K_2Cr_2O_7$) dissolved in glacial acetic acid. This reagent reacts with the residual H_2O_2 to form chromic acetate, which imparts a distinct color to the solution, allowing the quantification of catalase activity. The reaction was terminated by heating the mixture in a boiling water bath at 100°C for 10 minutes. This step ensured the complete reaction of the residual H_2O_2 with the coloring reagent, stabilizing the colored product. The resulting solution was then subjected to spectrophotometric analysis, with the absorbance measured at the wavelength of 240 nm to determine the catalase activity based on the amount of H_2O_2 decomposed during the initial reaction phase. This method provides a reliable and reproducible approach for quantifying catalase activity in biological samples.

Gene expression analysis
The expression study of ovarian aromatase (*cyp19a1a*) and liver vitellogenin (*vtg*) genes was carried out, per the standardized protocols, on *H. fossilis* (Chaube *et al.,* 2015).

a) Primer designing and validation:
The *cyp19a1a* (ovarian aromatase) and *vtg* (liver vitellogenin) gene-specific primers (listed in Table 3) were designed and validated for use with a SYBR Green-based qPCR kit (G2P, India), which employs a fluorescence-based dye to monitor DNA amplification in real-time (Chaube *et al.,* 2015).

Table 3: List of primers used in the gene expression study.

Accession No.	Gene	Primers (Forward→FP; Reverse→RP)	Amplicon
KJ619628.1	*cyp19a1a*	FP – CCATTTGGCTGTGGGCCAC RP – ATGGTGCTGACGGTGCAACC	172
GQ385192.1	*vtg*	FP – AGAGTTTGCTGCGGGTAAGA RP – TTGCAGCTGACTTTGACACC	105

a) Extraction of RNA and determination of its integrity:
The RNA samples were derived from the fish ovaries and liver from both the control and treatment (exposed to one-tenth of the LC_{50}96 hrs value of 4-tBP) groups. The ovarian and liver tissues (0.1 g) were homogenized in 1 mL of TRIzol reagent (Thermo Fischer Scientific, USA)

while ensuring thorough disruption of the tissue to release nucleic acids. Subsequently, the nucleic acids were separated through an organic phase separation process, a critical step for isolating high-purity RNA. Isopropanol was used in the RNA precipitation, followed by washing with ethanol to remove residual impurities. The precipitated RNA was air-dried to remove traces of solvents and was then dissolved in RNase-free water (Sigma, USA) to maintain RNA integrity (Chaube *et al.,* 2015). Lastly, the quality and concentration of the RNA samples were assessed using spectrophotometric methods (A260/280 ratio).

b) cDNA synthesis:
High-quality cDNA was synthesized to serve as the template for quantitative Polymerase Chain Reaction (qPCR) analysis (Chaube *et al.*, 2015). The extracted RNA samples were reverse-transcribed for this, following the manufacturer's protocol of a commercially available reverse transcription kit (G2P, India).

c) Real-time PCR:
The reaction setup and thermal cycling parameters followed the kit manufacturer's recommendations to ensure optimal amplification efficiency and specificity. Thermal cycling conditions typically included initial denaturation, annealing, and extension steps in the Quant Studio™ 5 Real-Time PCR System (Thermo Fischer Scientific, USA). Gene expression patterns were recorded and analyzed based on fluorescence data collected during the reaction, with particular attention paid to the cycle threshold (Ct) values for each gene of interest. Melt curve analysis was conducted post-amplification to confirm the specificity of the PCR products. This methodological approach enabled the robust quantification of gene expression and facilitated comparisons between control and treatment groups (Chaube *et al.,* 2015).

RESULTS AND DISCUSSION

Median Lethal Concentration (LC_{50}96 hrs)
The median lethal concentration (LC_{50}96 hrs) of 4-tBP was determined to be 27.8 mg/L in *H. fossilis* (Bloch, 1794) after a prolonged 96-hour exposure. The LC_{50}96 hrs value of 4-tBP-exposed fish (*H. fossilis*) was comparatively higher than that of the 4-tBP-exposed *Cyprinus carpio*, which exhibited an LC_{50}96 hrs value of 6.9 mg/L (Barse *et al.,* 2006), highlighting the stress-resistant capabilities of thefish model–*H. fossilis*.

On the other hand, the octylphenol- and nonylphenol-exposed *H. fossilis* (Bloch, 1794), respectively, exhibited comparatively lower LC_{50}96 hrs values of 0.9 mg/L (Srivastav *et al.,* 2025) and 1.6 mg/L (Gautam *et al.,* 2015). Based on this, it can be stated that 4-tBP exhibits lesser toxicity in the fish model (*H. fossilis*), whereas nonylphenol and octylphenol can exhibit increasingly higher toxicities.

Effect of 4-tBP on antioxidant enzyme activity
The activity of the enzymes, superoxide dismutase and catalase, was significantly decreased (as shown in Fig. 3A and 3B) in the *H. fossilis* (Bloch, 1794) exposed to a 2.8 mg/l

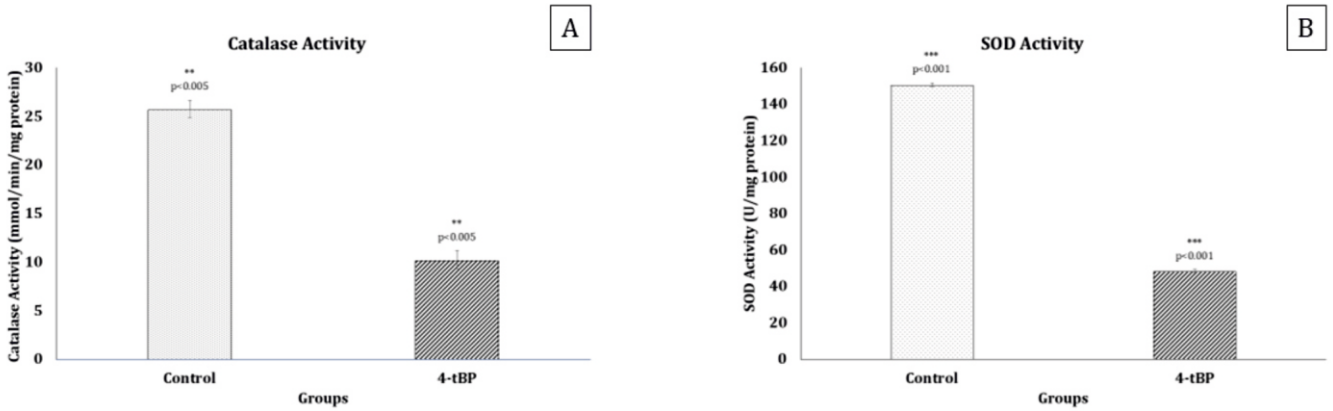

Fig. 3: Representation of antioxidant enzyme assay in *H. fossilis* (Bloch, 1794), against 4-tBP exposure: (A) Change in the SOD activity (U/mg protein);(B) Change in the Catalase activity (mmol/min/mg protein). Control: Untreated group; 4-tBP: Treated group with 4-tBP. Data are expressed as mean ± SD.

Based on the result, 4-tBP induces oxidative stress in fish, demonstrating increased production of reactive oxygen species (ROS). These ROS tend to overwhelm the fish's antioxidant defense system, which could have led to the reduction in the activity of these antioxidant enzymes (SOD and CAT). This reduction in antioxidant enzyme activity can disrupt the fish's ability to neutralize ROS, causing oxidative damage to vital organs such as the liver, kidneys, and gills. Additionally, phenols can interfere with DNA integrity and other cellular structures, further exacerbating the toxic effects. Similar trends in the reduction of SOD and CAT activity have also been reported in another study on the fish model– *H. fossilis* (Gautam and Chaube, 2021) along with *Salmo caspius* (Shirdel *et al.,* 2020) and *Silurus asotus* (Park, 2015), when exposed to 4-nonylphenol; whereas, in case of *Labeo rohita*, the activity of these enzymes reportedly decreased on the exposure of bisphenol (Ahmad *et al.,* 2025).

Effect of 4-tBP on ovarian aromatase (*cyp19a1a*) gene expression

A significant reduction of the *cyp19a1a* gene expression (Fig. 4A) in the ovary of the fish model– *H. fossilis* treated with 4-tBP was observed. The decrease in *cyp19a1a* expression in the ovaries of fish exposed to 4-tBP can be attributed to the endocrine-disrupting nature of the chemical.

The aromatase enzyme is critical in converting androgens into estrogens, essential for ovarian development and function (Guo *et al.,* 2024). The exposure of fish to alkylphenols may either inhibit the *cyp19a1a* activity or alter its transcriptional regulation, resulting in reduced estrogen production. This disruption can have cascading effects on ovarian physiology, including impaired follicular development and reduced reproductive capacity (Meier *et al.,* 2007). Alkylphenols, such as nonylphenol and octylphenol, also interfere with fish's normal hormonal signaling pathways (Lee *et al.,* 2024). These compounds can mimic or block the action of endogenous hormones, especially estrogen (E2), leading to disruptions in the synthesis and regulation of key enzymes like aromatase, which is encoded by the *cyp19a1a* gene (Sun *et al.,* 2021). The estrogen-mimicking effects of alkylphenols in teleost fish are extensively documented. Notable examples include the interaction of 4-nonylphenol with *Oncorhynchus mykiss* (Vetillard and Bailhache, 2006) and *H. fossilis* (Gautam and Chaube, 2021).

Effect of 4-tBP on liver vitellogenin (*vtg*) gene expression

A significant increase in the *vtg*mRNA expression was observed in the liver of the fish model– *H. fossilis*, treated with 4-tBP (Fig. 4B). The increase in *vtg* expression in the liver of fish exposed to 4-tBP can also be attributed to the endocrine-disrupting nature of the chemical. It is inferred that the compound (4-tBP) is structurally similar to that of natural estrogens, which can mimic how they bind to the liver estrogen receptors in the fish.

The upregulation of the *vtg* gene can be associated with higher concentrations of estrogen-mimicking 4tBP in the fish body, leading to higher synthesis of *vtg* protein that plays a significant role in oocyte maturation (Zhang *et al.,* 2011). Such a phenomenon has been reported in the females of *Cyprinus carpio* fish when exposed to nonylphenol (Amaninejad *et al.,* 2018). The vitellogenin biomarker upregulation was also seen in several other cases, such as octyl phenol-induced male *Danio rerio* (Lee *et al.,* 2024), nonylphenol-induced male *Oreochromis spilurus* (Abdulla Bin-Dohaish, 2012), and nonylphenol- and octylphenol-induced male *Oryzias latipes* (Chikae *et al.,* 2003).

CONCLUSION

The EDCs, undeniably, have been observed to present a serious threat to both aquatic organisms and human health. In fishes like *Heteropneustes fossilis* (Bloch, 1794), these contaminants interfere with normal hormonal functions, leading to severe reproductive challenges, developmental deformities, and eventually, a decline in the population. Likewise, in humans, exposure to EDCs has also been associated with various health concerns, including disruptions in

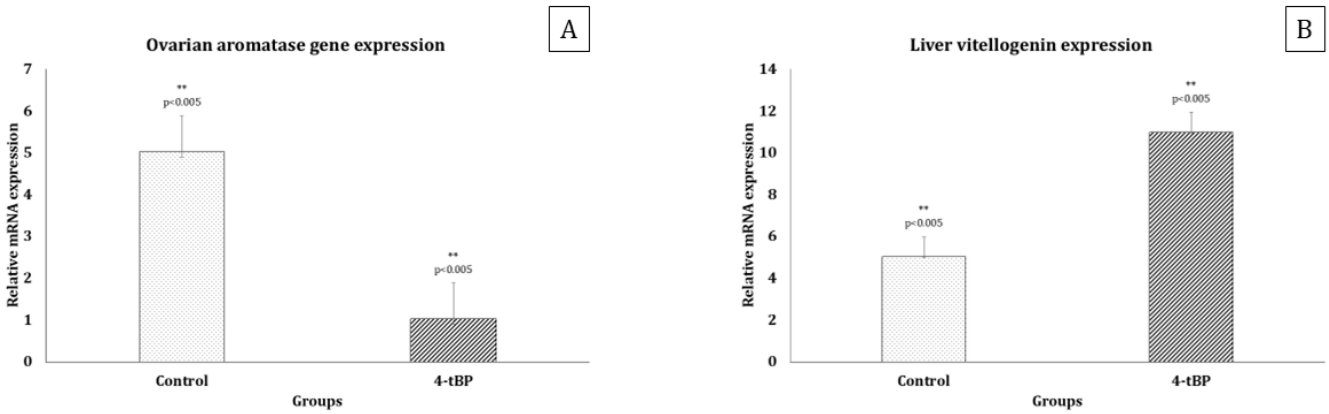

Fig. 4: Representation of gene expression profile in *H. fossilis*, against 4-tBP exposure: (A) Ovarian aromatase (*cyp19a1a*) gene; (B) Liver vitellogenin (*vtg*) gene. Control: Untreated group; 4-tBP: Treated group with 4-tBP. Data are expressed as mean ± SD.

the proper endocrine system, which leads to reproductive issues along with an increased risk of metabolic and neurological disorders. The widespread presence of EDCs in aquatic environments and food chains, it is crucial to implement effective strategies for their reduction. Strengthening environmental regulations, improving wastewater treatment processes, and promoting public awareness are essential to mitigating their impact. Additionally, advancing research on safer chemical alternatives and establishing long-term monitoring programs can help minimize exposure risks. Different collaborative and multidisciplinary approaches, including bioinformatics tools such as molecular docking, molecular dynamics simulations and multi-omics are necessary in understanding the behaviour of EDCs and thereby providing solutions to protect both the aquatic ecosystems and human well-being from the persistent dangers of these chemicals.

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REFERENCES

1. **Abdulla Bin-Dohaish E.J.** (2012). The effects of 4-nonylphenol contamination on livers of Tilapia fish (*Oreochromus spilurs*) in Jeddah. *Biological Research*. 45(1): 15-20. https://doi.org/10.4067/s0716-97602012000100002
2. **Ahmad S., Akmal H., Jabeen F. and Shahzad K.** (2025). Exposure to bisphenol S induces organ toxicity by disrupting oxidative and antioxidant defense system and blood physiology in *Labeo rohita* (Hamilton, 1822). *Fish Physiology and Biochemistry*. 51(2): 1-21. https://doi.org/10.1007/s10695-025-01467-1
3. **Amaninejad P., Sahafi H.H., Soltani M. and Shekarabi S.P.H.** (2018). Endocrine disrupting effects of 4-nonylphenol on plasma vitellogenin, reproductive system and histology in koi carp (*Cyprinus carpio*). *International Aquatic Research*. 10(3): 263-274. https://doi.org/10.1007/s40071-018-0203-8
4. **Barse A.V., Chakrabarti T., Ghosh T. K., Pal A. K. and Jadhao S.B.** (2006). One-tenth dose of LC50 of 4-tert-butylphenol causes endocrine disruption and metabolic changes in *Cyprinus carpio*. *Pesticide Biochemistry and Physiology*. 86(3): 172-179. https://doi.org/10.1016/j.pestbp.2006.03.006

5. **Chaube R., Rawat A. and Joy K.P.** (2015). Molecular cloning and characterization of brain and ovarian cytochrome P450 aromatase genes in the catfish *Heteropneustes fossilis*: Sex, tissue and seasonal variation in, and effects of gonadotropin on gene expression. *General and Comparative Endocrinology*. 221(15): 120-133. https://doi.org/10.1016/j.ygcen.2015.06.004

6. **Chikae M., Ikeda R., Hasan Q., Morita Y. and Tamiya E.** (2003). Effect of alkylphenols on adult male medaka: plasma vitellogenin goes up to the level of estrous female. *Environmental Toxicology and Pharmacology*. 15(1): 33-36. https://doi.org/ 10.1016/j.etap.2003.08.005

7. **Cui J., Shang X., Liu Y., Teng X., Zhou L. and Yan B.** (2025). Impact of 4-tert-Butylphenol on Inflammation and Glycogen Metabolism in *Cyprinus carpio* L via the miR-363/PKC Axis. *Environment & Health*. 3 (5): 539-550. https://doi.org/10.1021/envhealth.4c00242

8. **Fabbri L., Garlantézec R., Audouze K., Bustamante M., Carracedo Á., Chatzi L. *et al.*** (2023). Childhood exposure to non-persistent endocrine disrupting chemicals and multi-omic profiles: A panel study. *Environment International*. 173: 107856. https://doi.org/ 10.1016/j.envint.2023.107856

9. **Gautam G.J. and Chaube Radha** (2021). Modulation of antioxidant defence system in ovary and liver of catfish *Heteropneustes fossilis* (Bloch, 1794) and induction of atresia in ovary after chronic exposure of 4-nonylphenol. *Journal of Experimental Zoology India*. 24(1): 761-774.

10. **Gautam G.J., Chaube R. and Joy K.** (2015). Toxicity and tissue accumulation of 4-nonylphenol in the catfish *Heteropneustes fossilis* with a note on prevalence of 4-NP in water samples. *Endocrine Disruptors*. *3*(1): e981442. https://doi.org/10.4161/ 23273747.2014.981442

11. **Ghosh D. and Saha S.K.** (2022). Determination of the lethal concentration 50% (LC_{50}) of hexavalent Chromium in Nile Tilapia (*Oreochromis niloticus*). *Advances in Zoology and Botany*. 10(4): 123-131. https://doi.org/10.13189/azb.2022.100406

12. **Guo C., Zhang K., Li C., Xing R., Xu S., Wang D. and Wang X.** (2024). Cyp19a1a promotes ovarian maturation through regulating E2 synthesis with estrogen receptor 2a in *Pampus argenteus* (Euphrasen, 1788). *International Journal of Molecular Sciences*. *25*(3): 1583. https://doi.org/10.3390/ ijms25031583

13. **Hara A., Hiramatsu N. and Fujita T.** (2016). Vitellogenesis and choriogenesis in fishes. *Fisheries Science*. 82: 187-202. https://doi.org/ 10.1007/s12562-015-0957-5

14. **Hoang N.M. and Park K.** (2024). Applications of Tert-Butyl-Phenolic Antioxidants in consumer products and their potential toxicities in Humans. *Toxics*. 12(12): 869. https://doi.org/10.3390/toxics12120869

15. **Huang X.Z., Huang H., Chen H. and Wei Y.K.** (2024). Identification of endocrine-disrupting chemicals targeting key OP-associated genes via bioinformatics and machine learning. *Ecotoxicology and Environmental Safety*. 286: 117155. https://doi.org/10.1016/j.ecoenv. 2024.117155

16. **Kime D.E., Nash J.P. and Scott A.P.** (1999). Vitellogenesis as a biomarker of reproductive disruption by xenobiotics. *Aquaculture*. 177(1-4): 345-352. https://doi.org/ 10.1016/S0044-8486(99)00097-6

17. **Kumar Vishal, Shaw Ranjit and Chaube Radha** (2022). Survey on the potentialities of integrated fish farming, its economic and major constraints faced by the marginal and small farmers in Singrauli, Madhya Pradesh, India. *International Journal of Biological Innovations*. 4(1): 149-162. https://doi.org/ 10.46505/IJBI.2022.4117

18. **Lee J., Zee S., Kim H.I., Cho S.H. and Park C.B.** (2024). Effects of crosstalk between steroid hormones mediated thyroid hormone in zebrafish exposed to 4-tert-octylphenol: Estrogenic and anti-androgenic effects. *Ecotoxicology and Environmental Safety*. 277: 116348. https://doi.org/10.1016/j.ecoenv. 2024.116348

19. **Loganathan K., Tennyson S. and Arivoli S.** (2024). Triazophos toxicity induced

histological abnormalities in *Heteropneustes fossilis*, Bloch 1794 (Siluriformes: Heteropneustidae) organs and assessment of recovery response. *The Journal of Basic and Applied Zoology*. 85(1): 1-25. https://doi.org/10.1186/s41936-024-00373-x

20. **Meier S., Andersen T.E., Norberg B., Thorsen A., Taranger G.L. *et al.*** (2007). Effects of alkylphenols on the reproductive system of Atlantic cod (*Gadus morhua*). *Aquatic Toxicology*. 81(2): 207-218. https://doi.org/10.1016/j.aquatox.2006.12.002
21. **Nasreen S. and Borah G.A. (2023).** A Review on the use of *Heteropneustes fossilis* and *Clarias* magur in Assamese Traditional Medicine. *Asian Journal of Biological and Life Sciences*. 12(1): 18-21. http://dx.doi.org/10.5530/ajbls.2023.12.3
22. **Park K.H.** (2015). Alteration of hepatic anti-oxidant systems by 4-nonylphenol, a metabolite of alkylphenol polyethoxylate detergents, in Far Eastern catfish *Silurus asotus*. *Environmental Health and Toxicology*. 30: e2015006. https://doi.org/10.5620/eht.e2015006
23. **Prakash S. and Verma A.K.** (2015). Studies on different fish genera in Alwara lake of Kaushambi. *Bioherald: An International Journal of Biodiversity & Environment*. 5(1-2): 60-62.
24. **Prakash S. and Verma A.K.** (2016). Conservation status of fresh water fishes reported in Alwara Lake of District Kaushambi (UP). *International Journal of Zoology Studies*. 1(5): 32-35.
25. **PubChem** (n.d.). *PubChem*. PubChem. https://pubchem.ncbi.nlm.nih.gov/ [Accessed on 14/12/2024].
26. **Ratmuangkhwang S., Musikasinthorn P. and Kumazawa Y.** (2014). Molecular phylogeny and biogeography of air sac catfishes of the *Heteropneustes fossilis* species complex (Siluriformes: Heteropneustidae). *Molecular Phylogenetics and Evolution*. 79: 82-91. https://doi.org/10.1016/j.ympev.2014.05.009
27. **Salahshoori I., Mahdavi S., Moradi Z., Otadi M., Kazemabadi F.Z., Nobre M.A. *et al.*** (2024). Advancements in molecular simulation for understanding pharmaceutical pollutant Adsorption: A State-of-the-Art review. *Journal of Molecular Liquids*. 410:125513. http://dx.doi.org/10.1016/j.molliq.2024.125513
28. **Selvaraj K.K., Shanmugam G., Sampath S., Larsson D.J. and Ramaswamy B.R.** (2014). GC–MS determination of bisphenol A and alkylphenol ethoxylates in river water from India and their ecotoxicological risk assessment. *Ecotoxicology and Environmental Safety*. 99:13-20. https://doi.org/10.1016/j.ecoenv.2013.09.006
29. **Shirdel I., Kalbassi M.R., Esmaeilbeigi M. and Tinoush B.** (2020). Disruptive effects of nonylphenol on reproductive hormones, antioxidant enzymes, and histology of liver, kidney and gonads in Caspian trout smolts. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*. 232: 108756. https://doi.org/10.1016/j.cbpc.2020.108756
30. **Shukla S. and Prakash S.** (2024). Fish diversity of Dahaura Lake, Bahraich, U.P. *International Journal of Fisheries and Aquatic Studies*. 12(6): 142-146. https://www.doi.org/10.22271/fish.2024.v12.i6b.3099
31. **Srivastav A.K., Singh S. and Chaube R.** (2025). Acute toxicity of 4-Octylphenol on the histopathological changes of ovary in the freshwater Stinging Catfish *Heteropneustes fossilis*. *Journal of Experimental Zoology, India*. 28(1). http://dx.doi.org/ 10.51470/ez.2025.28.1.189
32. **Srivastava R., Tripathi S., Unni S., Hussain A., Haque S., Dasgupta N. *et al.*** (2021). Silybin B and cianidanol inhibit Mpro and spike protein of SARS-CoV-2: Evidence from in silico molecular docking studies. *Current Pharmaceutical Design*. *27*(32): 3476-3489. https://doi.org/10.2174/1381612826666201210122726
33. **Sun D., Chen Q., Zhu B., Zhao H. and Duan S.** (2021). Multigenerational reproduction and developmental toxicity, and HPG axis gene expression study on environmentally-relevant concentrations of nonylphenol in zebrafish. *Science of the Total Environment*.

764:144259. https://doi.org/10.1016/j.scitotenv.2020.144259

34. **Tripathi S. and Chaube R.** (2024). Endocrine Disrupting Chemicals: An Emerging Threat to Aquaculture and Sustainable Environment, in Dimensions of Life Sciences and Sustainable Development. 1st edn. Bhopal, M.P., India: Nitya Publications, pp. 11–26.
35. **Verma A.K.** (2016). Distribution and conservation status of catfishes in Alwara lake of District Kaushambi (UP). *International Journal on Environmental Sciences*. 7(1): 72-75.
36. **Verma A.K.** (2017). Distribution and conservation status of fishes reported from Muntjibpur pond of Allahabad (UP). *International Journal of Scientific World*. 5(1): 50-53.
37. **Verma A.K. and Prakash S.** (2016). Fish Biodiversity of Alwara Lake of District Kaushambi (U.P.). *Research Journal of Animal, Veterinary and Fishery Sciences*. 4(4): 5-9.
38. **Vetillard A. and Bailhache T.** (2006). Effects of 4-n-nonylphenol and tamoxifen on salmon gonadotropin-releasing hormone, estrogen receptor, and vitellogenin gene expression in juvenile rainbow trout. *Toxicological Sciences*. 92(2): 537-544. https://doi.org/10.1093/toxsci/kfl015
39. **Wang H., Liu J., Qiang S., Che Y. and Hu T.** (2024). 4-tert-Butylphenol impairs the liver by inducing excess liver lipid accumulation via disrupting the lipid metabolism pathway in zebrafish. *Environmental Pollution*. 356: 124385. https://doi.org/10.1016/j.envpol.2024.124385
40. **Zhang S., Wang S., Li H. and Li L.** (2011). Vitellogenin, a multivalent sensor and an antimicrobial effector. *The International Journal of Biochemistry & Cell Biology*. 43(3): 303-305. https://doi.org/10.1016/j.biocel.2010.11.003