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Environmental Consequences of Benzo[a]pyrene in Fish

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are widespread contaminants in aquatic environment. Benzo[a]pyrene (B[a]P) is one of the prototypes of PAHs which is formed during the incomplete burning of organic materials. High-performance liquid chromatography (HPLC) and Gas chromatography mass spectrometry (GC-MS) are the appropriate tool to assess B[a]P concentration in fish. This study reveals that the B[a]P accumulation in fish which alters ROS formation, antioxidants activity, MN induction, histological lesions, and molecular mechanisms.

Keywords: Polycyclic aromatic hydrocarbons, Benzo[a]pyrene, Oxidative stress, Genotoxicity, Fish

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are universal pollutants in aquatic habitats. The major portion of PAHs arrives into water bodies from land-based runoff or atmospheric deposition. Though PAH levels deteriorated in urban watersheds from 1970s to 1980s because of decrement in coal burning and industrial discharges (Guntupalli et al., 2016; Parmar et al., 2020; Yuan et al., 2021). But the last time period has formed new increases in PAH accumulation in aquatic organisms due to automobile practice linked through urban extension (Saha et al., 2009) or diesel burning by heavyweight vehicles (Baum et al., 2016). Furthermore,

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new studies address that petroleum hydrocarbons produced from oil spills can easily persist in aquatic sediments, plants and animals for years or longer (Vanzella et al., 2007).

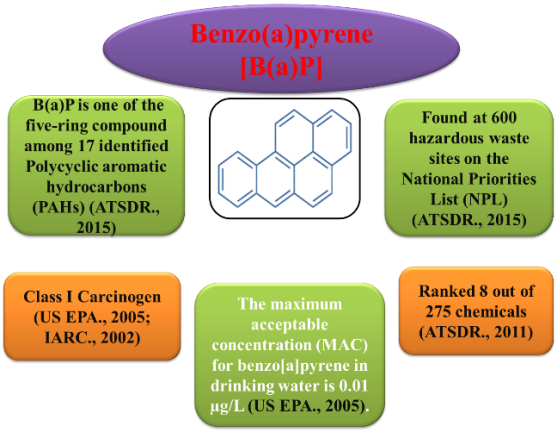

Figure 1: B[a]P as a toxic substance.

B[a]P is one of the carcinogenic petroleum chemicals with two or more fused aromatic rings which is widely dispersed in the environment (Harris et al., 2020). The production or utilization of B[a]P is not done commercially, but is generally available in the environment. It is formed primarily as outcome of pyrolytic events, particularly the incomplete incineration of organic resources in industrial and various human actions like processing of crude oil and coal, ignition of natural gas for culinary and wood burning (Hamilton et al., 2021; IARC, 2014; Le Bihanic et al., 2014). Additional human actions that result in B[a]P release include the garbage burning, road traffic by vehicles, tobacco and cigarette smoking, and ingestion of charcoal roasted and smoked foods (Ramesh and Archibong, 2011; Sen and Field, 2013). B[a]P binds to small elements in the air and is easily diffused into the environment through the air. B[a]P can then be inhaled or deposited in the environment on plants, soil, and water bodies (Guo et al., 2021; Jabeen et al., 1234; WHO, 2003). Aquatic animals and plants may then come in contact with B[a]P. B[a]P, and its metabolites can persist in the aquatic environment for many years. After the exposure of B[a]P in the residues of ponds, lakes, and waterways, it accumulates in the organs of aquatic invertebrates and vertebrates (Batel et al., 2018; Fanali et al., 2018; Huang et al., 2014). Like other PAHs, B[a]P is mainly toxic to aquatic organism in the occurrence of UV light, increasing health risk to the animals in unshaded or shallow environments (Sen and Field, 2013).

Fishes eagerly take up lipophilic organic substances such as B[a]P from the aquatic environment, with various physiological, cellular and molecular effects (Albornoz-Abud et al., 2021; Honda and Suzuki, 2020). Fish are able to break down xenobiotics, so there is little

accumulation of B[a]P in fish (Scott and Jones, 2000). However, B[a]P acts as a genotoxicant in aquatic organisms, causing many biochemical changes most studied in fish, and the studies on effects of B[a]P on physiological perturbations of fish are comparatively limited. Furthermore, studies are needed to explore the toxic effects of B[a]P in fish.

The quantification of physiological, biochemical, and molecular constraints is a diagnostic implement regularly used in aquatic toxicology and biomonitoring (Dong et al., 2019; Javed and Usmani, 2019). Micronucleus (MN) test is a very reliable tool to asses the genotoxic potential of any xenobiotics (Awasthi et al., 2019). MN formation in erythrocytes of fish by single and double strands break of DNA, and may also a result of the inappropriate DNA repair or failed DNA repair mechanism (Fenech, 2002). Assessment of oxidative stress reveals the redox state of the cell. To deal with enhanced Reactive oxygen species (ROS) in cell, defence mechanism works in which enzymatic- superoxide dismutase (SOD), catalase (CAT), Glutathione reductase (GR), and non-enzymatic antioxidant- reduced glutathione (GSH) assist to maintain the homeostasis in cell (Jifa et al., 2006). The oxidative stress generated DNA damage and alter different molecular mechanisms such as apoptosis, and autophagy in fish. The present study comprises of different techniques for assessing B[a]P toxicity and its deleterious effects in fish.

Materials and methods

Estimation of B[a]P in fish

The B[a]P estimation in fish samples can be done by using High-performance liquid chromatography (HPLC) and Gas chromatography mass spectrometry GC-MS techniques:

Estimation through HPLC

In order to quantify B[a]P accumulation, fish samples can be obtained using the Soxhlet extraction method with a mixture of acetone and dichloromethane (1:1). The obtained extracts will be dried by anhydrous sodium sulfate and then concentrated through a rotary evaporator. The concentrated extract will then be diluted with 10 mL of hexane and then further concentrated with 2 mL of hexane. The prepared extracts will be used for the analyses of B[a]P in fish tissues using water–acetonitrile solvent organization on HPLC equipped with pump and UV–vis detector. C-18 column will be preferred for the B[a]P detection. The flow rate can be adjusted to 1 mL/min. The B[a]P can be recognized by retention time compared with reference to the B[a]P standards (Malik et al., 2008).

Estimation through GC-MS

The lyophilized fish samples will be used for B[a]P determination through GC-MS. For GC-MS analysis, carrier gas-Helium and the column pressure-10 psi will be maintained to provide an estimated flow rate of 1 ml/min. The injector lines at 290 °C and transfer lines at 250 °C will be maintained. The temperature of columns will be maintained at °C for 4 min, ramped at 300 °C at a range of 10 °C/min. The mass spectrometer will be employed in electron ionization manner and all the spectra will be obtained with a huge range of m/z 50–400 and automatic gain control (AGC) (Giri et al., 2013).

Experimental design on fish

Freshwater live of test model will be collected and brought to the laboratory in an aerated medium (Trivedi et al., 2021). During the acclimation, fish will be kept in ventilated glass aquaria and fed with food pellets twice a day. All the necessary physiochemical properties (pH, Temperature, DO, Alkalinity, and Hardness) of aquaria water will be maintained. After the 15 days acclimatization, healthy specimens will be randomly distributed in all the experimental aquaria. The experimental groups were selected in triplicates. Sampling will be done at estimated time interval. Test parameters- Micronucleus test, ROS, biochemical activity (SOD, CAT, GSH and GR), histopahology and transcriptional analysis can be performed at each sampling duration by following the protocol of Trivedi et al., (2022).

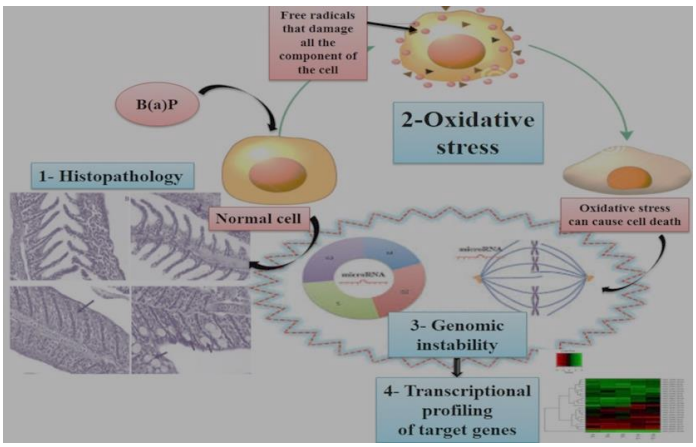

Figure 2: Effects of B[a]P.

B[a]P estimation and its detrimental effects in fish

The measurement of B[a]P and other PAHs in surface water, sediments and fish was performed by using HPLC from eight different locations of the river Gomti (India) (Malik et.

al., 2010 and Malik et al., 2008). Similarly, Palanikumar et al., 2012 investigated the accumulation of B[a]P through GC-MS after its sub-lethal exposure in fish, *Chanos chanos*. B[a]P when enters into the fish, different metabolites formed which cause ROS production via redox cycle. Qi and Tang, (2020) also examined the ROS production after the 96h exposure of B[a]P in mussel *Mytilus coruscus*. Increased ROS production may cause extreme oxidative stress, to deal with this oxidative stress an antioxidant defense system works with different enzymatic (SOD, CAT and GR) and non-enzymatic (GSH) antioxidants. Antioxidant-SOD acts as first line defence against oxidative stress caused due to B[a]P and catalyzes excess superoxide radicals into H_2O_2 (Gravato and Guilhermino, 2009; Guo et al., 2021). SOD induction was also found in different fish species exposed to waterborne contaminant B[a]P (Dellali et al., 2021; Qi and Tang, 2020). Further, CAT enzyme breaks H_2O_2 into water and augmented CAT activity designates the higher concentration of H_2O_2 in cell. Rodrigues et al., (2022) also measured the increased activity of CAT enzyme in tissues of *Scrobicularia plana* after B[a]P exposure. GR maintains the redox potential of cell by regenerating GSH from GSSG. Increased level of GR was documented in tissues of mussel *Mytilus coruscus* exposed to B[a]P (Qi and Tang, 2020). A non-enzymatic antioxidant-GSH was also estimated in different body parts of fish (Jifa et al., 2006; Santos and Bueno, 2020).

Studies clarified that the B[a]P was found genotoxic for the fish. It upsurges the frequency of MN formation in cells. After the acute exposure of B[a]P, raised MN frequency were detected in rainbow trout and common carp (Kim and Hyun, 2006). Another study established that B[a]P raised the MN formation in milkfish-*Chanos chanos* after 96 h of experimental duration (Palanikumar et al., 2012).

Histopathological results may help to examine a range of anomalies in the liver and kidney tissues of fish. Numerous studies have been repored the different histological modifications tissues of fish after B[a]P exposure (Briaudeau et al., 2021; Carlson et al., 2004; Esmaeilbeigi et al., 2021; Woo, 2022).

Transcriptional analysis helps to quantify the expression of specific genes. Various studies have been done on mRNA expression in different organs exposed to B[a]P. In a study, up-regulation of tumor suppressor gene-p53 was found involved in autophagy by down-regulating the mTOR gene after B[a]P exposure which is investigated by Lin et al., (2016) and Sforzini et al., (2017).

Indeed, more studies need to be performed to evaluate the toxic ability of B[a]P in different tissues of fish based on MN, antioxidant enzymes, transcriptional analyses, and other related biomarkers.

Conclusion

The present study reveals the toxic potential of B[a]P in fish and its deleterious effects on genotoxicity, oxidative stress, histopathological impressions and transcriptional analysis in fish. The study also discloses the assessment of B[a]P accumulation and its genotoxic ability in fish. B[a]P is also able to accumulate in fish and cause oxidative stress by producing ROS. Thus, the study helps in understanding the toxic impact of B[a]P in fish and performing the future studies on aquatic creatures. Increasing concentration of B[a]P causing major health risk to aquatic organisms.

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