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Effects of Bisphenol A on the liver histopathology of Indian major carp, *Labeo rohita* (Hamilton, 1822).

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ABSTRACT

Endocrine disruptors are produced naturally as well through various anthropogenic activities. Both of these types are capable of producing a certain endocrine disruption in the physiology of organisms dwelling in that environment. A multitude of study revealed and recorded the occurrence of Endocrine Disrupting Compounds (EDCs) in Indian freshwater which were at potentially toxic levels. Bisphenol A (BPA) is one such compound whose accumulation in the freshwater ecosystem causes disruption in the endocrine physiology of the fishes as it simulates estrogenic activities. Liver is one of the major organs in fish that aids in detoxifying the system. The current study aims to understand the detrimental effects of Bisphenol A on the liver histopathology of *Labeo rohita* a commercially available food fish.

Keywords: **Endocrine disruptors, Bisphenol A, liver histopathology, detrimental effects.**

INTRODUCTION

Bisphenol A is one of the derivatives of Bisphenols, a group of synthetic compounds used in manufacturing of polycarbonate plastics (Ohore et al. 2019). It is known to act as an endocrine disrupting compound as it potentially affects the endocrine system by mimicking estrogenic activities. BPA is known to have a weak estrogenic activity near to 1/1000- to 1/10000- fold of 17β-estradiol (E2); additionally the molecules interact with classical estrogen receptors alpha (ER-α) and beta (ER-β) or with non-classical G-protein coupled receptor 30 (GPR30) (H. Guo et al. 2010). *Labeo rohita* (Hamilton, 1822) commonly called as Rohu is an Indian major carp. It is extensively found in South Asian freshwaters. It is an omnivore and is widely used in industries of aquaculture and fisheries. In India, it is consumed as a delicacy in the eastern, western, central and northern regions. A survey across areas around Mumbai revealed that the fauna found inside the freshwater ecosystem are highly affected because of the pollutants present in them. As *Labeo rohita* (rohu fishes) are an important part of the local diet, there is a high probability in the long run that these fishes could have an adverse effect on the human health since they are caught and distributed to major fish markets in the city. The water sampling reports revealed the presence of BPA in trace amounts from two major sampling sites around the city. This evidently proved that the fishes in these waters consistently get exposed to an array of toxicants, one of which is Bisphenol A. Thus it was imperative to analyze its histopathological effects on the liver to evaluate the damage caused by this compound as the liver is one of the major organs that aids in detoxifying the system.

MATERIAL AND METHODS

Analysis of pollutants in water from research sites: After careful survey, water sampling sites were finalised and water sampling was done. Samples were collected from two different locations from Thane district namely Ulhas river, Wehele and Thane Creek, Thane region respectively. The latitudes and longitudes of each location was recorded to mark the precise location of sampling sites. Locations were finalised based on the presence of Rohu fishes, which were abundantly found in these waters. Evidence confirmed the fishing sites near these locations, where this species is caught and sold in local markets. The main objective of water sampling was to analyse the samples to detect the presence of Endocrine Disrupting compounds.

Sample collection and feed: *Labeo rohita* was procured from Aarey Fish Farm, Goregaon, Mumbai and for experimental purpose, the sub-adult stage of the fishes (yearlings) were finalised for study and observation. The approval for this study was sought from the CPCSEA. Experiments were conducted for 30 days of which 8 days was the Acclimation phase and 21 Days the Experimental phase. The feed consisted of rice bran and groundnut oil cakes crushed into a fine powder in a ratio of 1:1 and was given as 2% of total body weight of the fishes present in each tank. Fishes received this feed for 8 days of the acclimation phase and subsequently, once the fishes were acclimatised to this feed, from day 9, the exposure phase was conducted by administering a dose of Bisphenol A to the feed. Initial exposure comprised of a mild dosage, thereafter the dose was gradually increased throughout the Exposure phase of 21 days. Fishes from the Controlled Tanks continued to receive the same feed without the dose. The initial dose was confirmed with respect to the previous LC_{50} values of Bisphenol A.

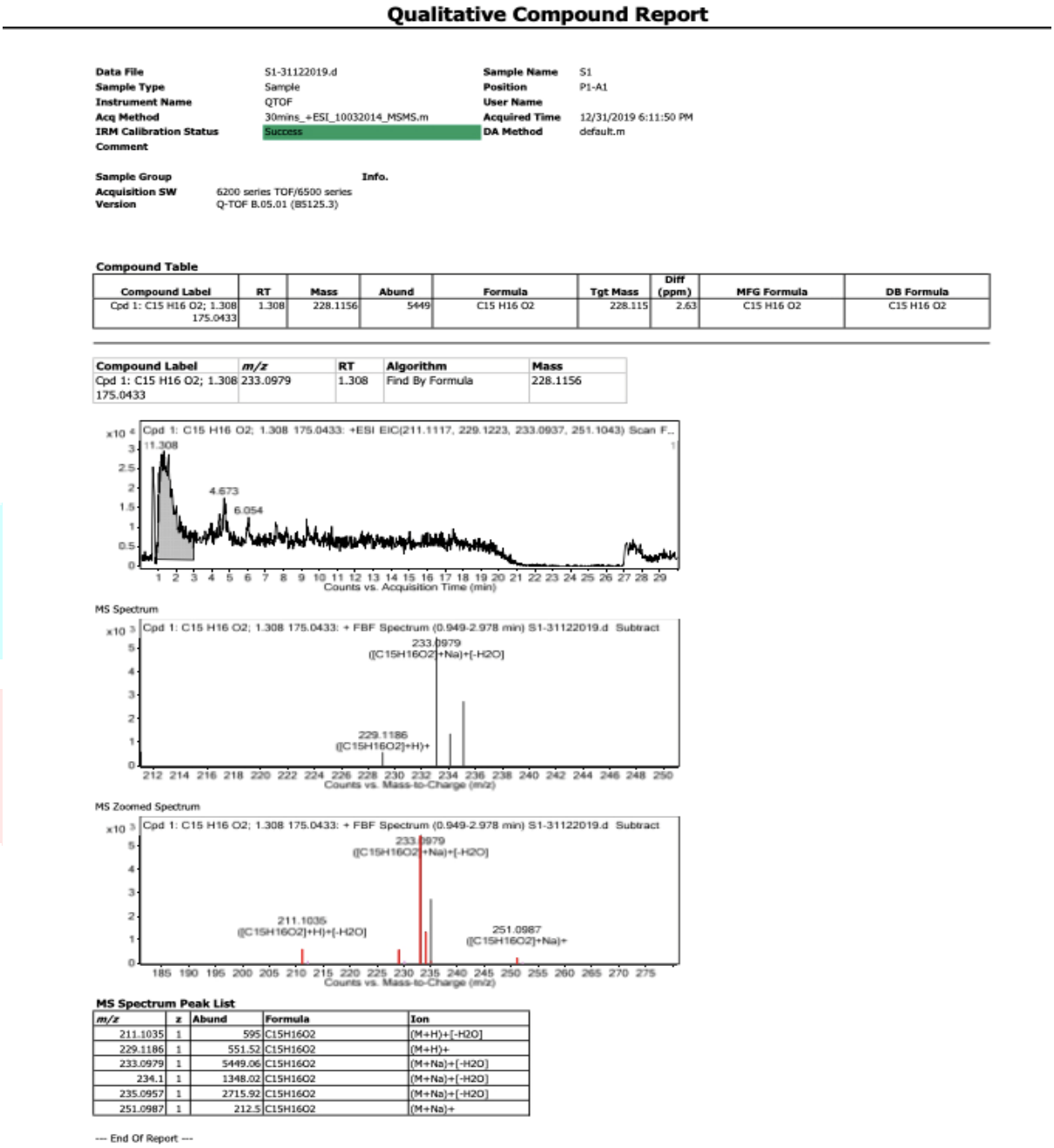
Histological examination: Histological analysis was performed on tissues affected with BPA. The organs were carefully dissected from the fishes after completion of the exposure period or post mortality. Liver lobes were separated from under the ventral region of the heart. The tissue samples were preserved in formalin and dehydrated by treating with ascending grades of alcohol. Liver tissues were cleared with xylene and impregnated with wax. All sections of the liver tissue were cut 5µ thick. They further underwent systematized staining with Hematoxylin and Eosin (H & E) stains. All slides were carefully examined after staining and photographs were recorded with a high resolution camera from the light microscope at power 10X and 45X respectively.

OBSERVATIONS AND RESULTS

The water samples were processed under HR-LCMS (High Resolution Liquid Chromatography Mass Spectrometry) in order to detect the target endocrine disrupting compounds in the sample. This technique combines High Performance Liquid Chromatography (HPLC) a powerful analytical and separation technique along with mass spectroscopy, which is a powerful analysis and detection technique. A Qualitative Compound Report (Fig A) demonstrated various array of compounds found in both samples of which Bisphenol A was detectable in trace amounts. Observations from hepatic tissues of *Labeo rohita* from control group indicated normal cell morphology. The portal vein was observed to be lined with epithelial cells along with intact bile duct and structurally diverse hepatocytes were arranged in cords and surrounded by cytoplasm and blood sinusoids as observed in Fig. 1, Fig. 2 and Fig. 5. Control group hepatocytes consisted of a centrally located nucleus with no sign undergoing vacuole formation along with a an intact central vein (Fig. 3). Overall toxicity caused by exposure to Bisphenol A was demonstrated during the three-weeks of experimental phase. After commencement of BPA exposure, fish liver exposed to 3mg BPA exhibited formation of a few vacuoles. Major histopathological alterations with an increase in dosage were observed in hepatocytes of fish exposed to over 18mg BPA. LC_{50} value for BPA was attained at 46ppm. The alterations in liver structure involved major loss of normal architecture of hepatocytes, severe hemorrhage and necrosis around vital organelles that carry important functions. Toxic effects of BPA exhibited severe lipid vacuolization of hepatocytes as seen in Fig. 4a and Fig. 4b. Cell lysis along with aggregation of massive necrotic tissues induced total disruption of

the portal vein and the central vein (Fig. 1a, Fig. 1b, Fig. 1c, Fig. 3a, Fig. 5a, Fig. 5b). Severe damage to the bile ducts and blood sinusoids was also observed as a detrimental effect as it can be observed in Fig. 2a and Fig. 2b.

TABLES AND FIGURES:



Qualitative Compound Report

Data File	S1-31122019.d	**Sample Name**	S1
Sample Type	Sample	**Position**	P1-A1
Instrument Name	QTOF	**User Name**	
Acq Method	30mins_+ESI_10032014_MSMS.m	**Acquired Time**	12/31/2019 6:11:50 PM
IRM Calibration Status	Success	**DA Method**	default.m
Comment			

Sample Group **Info.**
Acquisition SW Version 6200 series TOF/6500 series Q-TOF B.05.01 (B5125.3)

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula
Cpd 1: C15 H16 O2; 1.308 175.0433	1.308	228.1156	5449	C15 H16 O2	228.115	2.63	C15 H16 O2	C15 H16 O2

Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C15 H16 O2; 1.308 175.0433	233.0979	1.308	Find By Formula	228.1156

MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
211.1035	1	595	C15H16O2	(M+H)+[-H2O]
229.1186	1	551.52	C15H16O2	(M+H)+
233.0979	1	5449.06	C15H16O2	(M+Na)+[-H2O]
234.1	1	1348.02	C15H16O2	(M+Na)+[-H2O]
235.0957	1	2715.92	C15H16O2	(M+Na)+[-H2O]
251.0987	1	212.5	C15H16O2	(M+Na)+

--- End Of Report ---

Fig A: Qualitative compound report indicating the detection of BPA in water sample analysis.

MICROGRAPHS OF LIVER SECTIONS REVEALING DAMAGE CAUSED BY INDUCTION OF BPA:

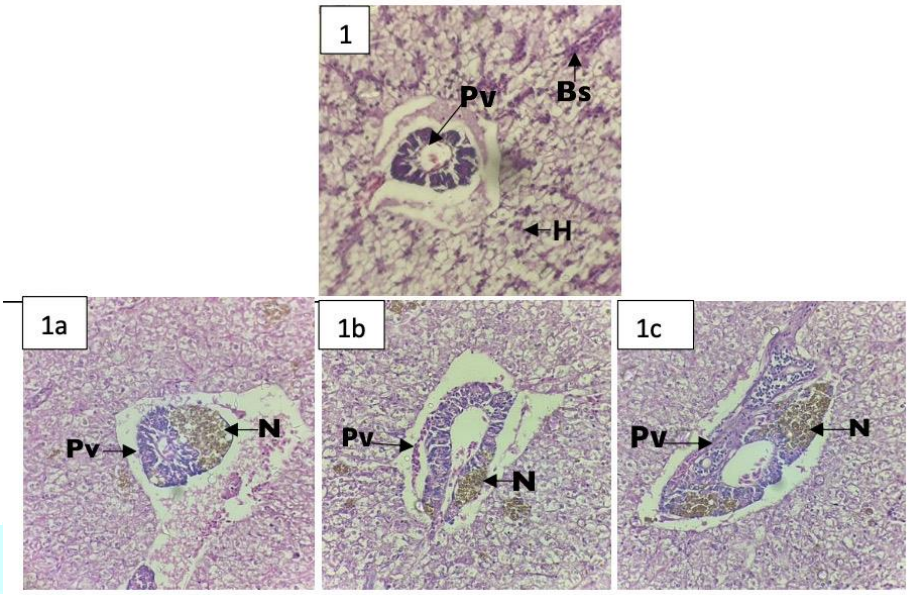


Fig 1: T.S of Control Liver in Rohu fish showing healthy hepatocytes (H), Portal Vein (PV) and Blood Sinusoids (Bs). [H x E stain; 40x]
Fig 1a: Effects of BPA, Necrosis (N) observed around Portal Vein (Pv) with disintegrating nests of cells. [H x E stain; 40x]
Fig 1b: Effects of BPA, Necrosis (N) observed around Portal Vein (Pv) with disintegrating nests of cells. [H x E stain; 40x]
Fig 1c: Effects of BPA. Necrosis (N) observed around Portal vein (Pv). [H x E stain; 40x]

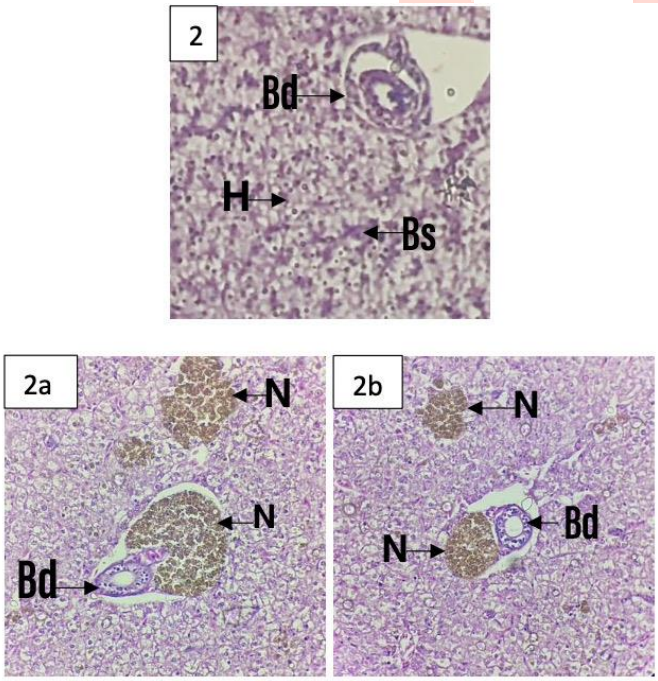


Fig 2: : T.S of Control Liver in Rohu Fish showing healthy Hepatocytes (H) and blood sinusoids (Bs), Bile duct (Bd). [H x E stain; 40x]
Fig 2a: Effects of BPA, Necrosis (N) around Bile duct (Bd) with disintegrating cells. [H x E stain; 40x]
Fig 2b: Effect of BPA showing (N) around the Bile duct (Bd) around cluster of disintegrating cells. [H x E stain; 40x]

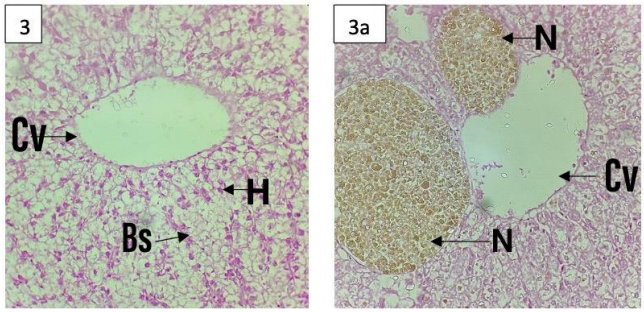


Fig 3: Section of liver in Control fish showing normal morphology of Hepatocytes (H), Central Vein (Cv) and Blood Sinusoids (Bs). [H x E stain; 40x]
Fig 3a: Effects of BPA. Necrotic structures (N) around the Central Vein (Cv). [H x E stain; 40x]

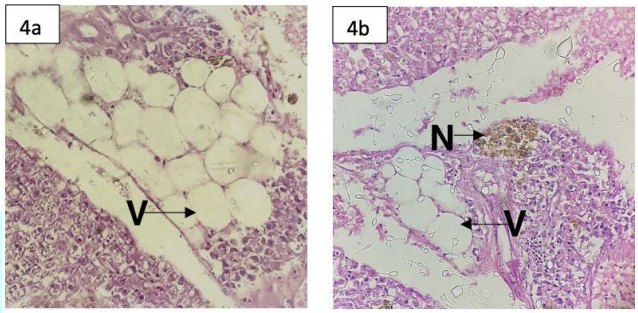


Fig 4a: BPA affected fish liver showing heavy Vacuolation (V). [H x E stain; 40x]
Fig 4b: BPA affected fish liver showing Vacuolized structures (V), Necrosis (N). [H x E stain; 40x]

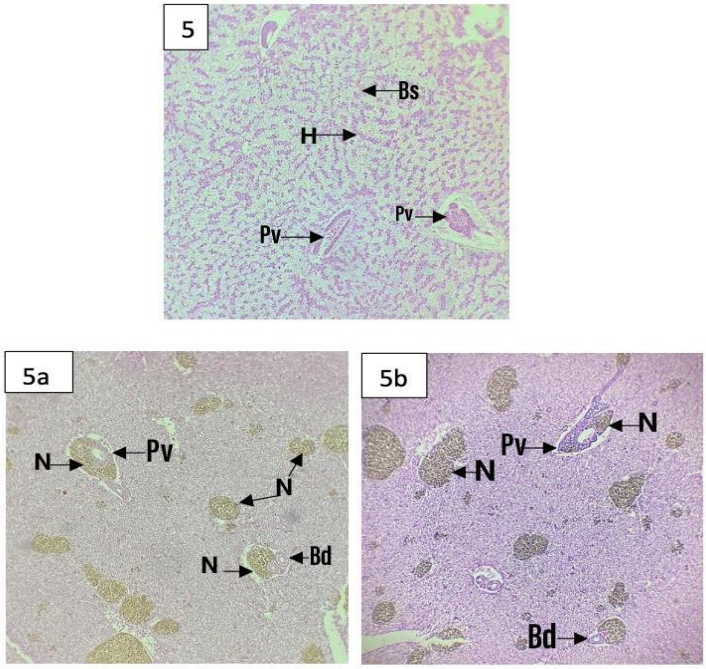


Fig 5: T.S of Control Liver in Rohu Fish showing healthy Hepatocytes (H) and blood sinusoids (Bs), Normal Portal Vein (Pv) [H x E stain; 10x]
Fig 5a: Effects of BPA, Necrosis (N) around Bile duct (Bd) and Portal vein (Pv). [H x E stain; 10x]
Fig 5b: Effect of BPA showing Necrotic tissues(N) around the Bile duct (Bd) and Portal Vein (Pv). [H x E stain; 10x]

DISCUSSION

According to Genten et al. 2009 the teleost fish liver and its cellular components mainly the hepatocytes are structurally similar to mammals but unlike mammalian liver, the liver lacks true portal tracts and the fish hepatocytes are not arranged in lobes. Studies on histopathological effects of Bisphenol A on liver of *Heteropneustes follisilis* carried by Roy et al. 2011, suggested that the groups treated with BPA indicated hepatocellular degeneration and pycnotic nuclei were evident along with fibrosis in the liver. According to Faheem et. al, who worked on the histopathology of liver in *Catla catla* exposed to BPA indicated a few ruptured hepatocytes and ruptured central vein, whereas the higher

concentrations of BPA showed dilated and congested central vein along with degeneration of hepatocytes, necrosis and vacuolization. Infiltration of massive number of macrophage was also observed.

In the current studies, *Labeo rohita* treated with BPA exhibited certain impairments in hepatocytes, portal vein and central vein, along with damage leading to necrotic tissues and abnormal vacuolation due to distinct aggregation of glycogen particles. The present work indicates similar observations with respect to the work of Faheem et. al. 2016. Although the results observed in the current research is at par with the work of Faheem et al. 2016, elucidated effects of BPA on the liver in food fish *Labeo rohita* is first of its kind. Thus it can be concluded that BPA is a potential hepatotoxic agent in fishes.

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