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# BISPHENOL A (BPA) INDUCEDHISTOPATHOLOGICAL AND BIOCHEMICAL ALTERATIONS IN THE LIVER AND KIDNEY OF STINGING CAT FISH *HETEROPNEUSTES FOSSILIS*

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**ABSTRACT**

The freshwater fish, *Heteropneustes fossilis* was exposed to various sublethal concentrations of 0.714 mg/L ($1/10^{th}$), 1.428 mg/L ($1/20^{th}$) and 2.142 mg/L ($1/30^{th}$) of Bisphenol A(Group II, III and IV) for 28 days and was studied for several hepatic and renal biochemical and histopathological constraints. After termination of the experiment (28days), the fish liver and kidney were taken out and analyzed for total protein, plasma glucose, plasma creatinine AST and ALT (SGPT and SGOT). Results clearly shown that the treatment of sub lethal concentrations of BPA induced significant alterations in the structural design of both liver and kidney. The total proteins content in both the tissues was decreased significantly. However, plasma glucose and creatine content of the fish of all treated groups increased significantly as compared to control sample. Sub lethal concentrations of BPA treatment induced severe damages in both liver and kidney of the fish in dose dependent manner. The reduction in total protein content in both the tissues of all experimental groups may be because of renal excretion or impaired protein synthesis or due to damage of hepatocytes. The present study confirms that sub lethal concentrations of BPA induced various histopathological and metabolic anomalies in *H.fossilis*. Overall, our study suggests that the incorporation of sublethal concentrations of BPA can alter liver and renal function in *H.fossilis* and permits further research.

**KEYWORDS:** BPA, *H. fossilis*, Biochemical and histopathological parameters, liver, kidney

## INTRODUCTION

BPA has become a public health concern due to its extensive and continuous exposure through food and drinking water. Identification of susceptible aquatic species to various toxic chemicals an essential factor in the environmental risk assessment programmes. The mounting waste product burden and in excess and misuse of the water resources significantly decreases the carrying capacity of ecosystem. Accordingly, the biological communities including fish, inhabiting them, eventually face the dual stress applied on the water bodies (Reddy, 2017; Bavinck, 2018). The entry of these contaminants into aquatic systems comprises a major threat to water chemistry and biological characteristics of the aquatic ecosystems. The exposure of fish to various contaminants provokes a number of alterations in fish physiology at molecular level particularly reproductive organs, liver and kidney. Many researchers have evaluated the effects of industrial chemicals on various aquatic species independently by integrating histopathological, biochemical and molecular biomarkers on different species (Reddy, 2012; Reddy and Kusum, 2013; Patneedi and Prasadu, 2015; Reddy, 2017; Reddy, 2013; Kumari and Khare, 2018). Under stable environmental conditions, susceptibility of living organisms to toxic substances varies among individuals and within an individual over time because of intrinsic factors (species, gender, age, development, maturation). Metabolic enzymes like Aspartate aminotransferase (AST, EC 2.6.1.1) and alanine aminotransferase (ALT, EC 2.6.1.2) are found chiefly in the liver, but also found in red blood cells, heart, muscle and kidneys. AST or ALT levels are a valuable support predominantly in the analysis of liver disease (Huang, 2006).Creatinine is a by-product of muscle metabolism and a reliable biomarker of kidney function. Elevated creatinine level indicates weakened kidney function (Kulkarni and Pruthviraj, 2016).

Bisphenol A (BPA) is a universal organic synthetic, colourless compound with the chemical formula $(CH_3)_2C(C_6H_4OH)_2$.This chemical can leach into food from containers lined with epoxy resin coatings and from polycarbonate plastic products. Warming the plastic implements in a microwave or keeping hot fluids in plastic containers increases the leaching of BPA into liquids (https://www.niehs.nih.gov/health/topics). Klecka *et al.* (2009), confirmed the presence of BPA in surface water and sediment in the US and Europe and this substance may perhaps accumulate in our waters and harm fish and other organisms (Heinonen, J. *et al* 2002). The possible destructions caused by BPA is a topic of scientific debate and that further investigation was a priority because of the association between BPA exposure and adverse humans health effects on reproductive, developmental effects and metabolism. Most of the studies are restricted to examine the biochemical and physiological changes of BPA but very little consideration was given to

  

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make a comparative analysis of biochemical and histopathological studies. For that reason, the present study is aimed to investigate the biochemical and histopathological alterations in liver and kidney of stinging cat fish *H. fossilis* exposed to various sublethal concentrations of Bisphenol A.

## MATERIALS AND METHODS

### Experimental Design:

Healthy adult fish irrespective of sex were procured from local market during winter season of 2016 (weight of 24.09 ± 1.42g and total length of 17.09 ± 0.24) and were acclimatized to the laboratory settings for 15 days in aquaria of 250L capacity. Afterwards, the fishes were divided into four exposure sets containing 10 fishes in each aquarium (control and BPA treated) at the Department of Zoology, Government PG College, Ratlam, M.P. Bisphenol-A of 99.8% pure was purchased from Chemex Organochem Private Limited, Mulund West, Mumbai, Maharashtra (India). The fishes were exposed to different sublethal concentrations of BPA (0.714 mg/L ($1/10^{th}$), 1.428 mg/L ($1/20^{th}$ and 2.142 mg/L ($1/30^{th}$) i.e. (Group II, III and IV) for 28 days using ten fishes in each aquarium. The sublethal concentrations were selected based on earlier published LC50 value of BPA for *H.fossilis* (Roy, 2011).

Dosages of BPA were given on every alternate day basis. During the period of experiment all the experimental and control sets of fishes were fed with commercial food. Washing, reintroducing with freshwater and feeding activities were continued regularly on every alternative day. An electronic $O_2$ monitor was fitted for continuous monitoring of $O_2$ levels. All aquaria were placed in similar and natural environmental conditions. After the termination of experiment, on 29th day, four fishes from each treated aquarium were handpicked and dissected for liver and kidney. Immediately the tissues were washed in 0.75% saline solution, blotted with tissue paper, kept in Teflon tubes, and finally stored at $-20^0$C for later analysis.

### Collection of blood sample:

The experimental fish on $29^{th}$ days were placed belly upwards and blood samples acquired from the caudal perforation with the help of a heparinized 2cm disposable plastic syringe. The usage of plastic syringe is an essential safety measure as fish blood when contact with glass results in reduced coagulation time. The spots elected for puncture (about 3-4cm from the genital opening) was smeared dry with cotton to avoid contamination with mucus. The needle was injected at right angle to the vertebral column of the fish and was softly extracted during penetration. Then after, the needle was smoothly pushed down until about 2 ml blood enters into the needle from caudal blood vessel. Afterwards the needle was withdrawn slowly and the blood was smoothly shifted into plastic vessels.

### Tissue Preparation:

Both the liver and kidney were taken out from the freezer and soaked with tissue paper. The tissues were weighed maximum up to 0.1 g and homogenized in 2 mL of 0.5 M (pH 7.4) Tris-HCl buffer by using *REMI* Lab *Homogeniser* (RQ-127A/D) and finally centrifuged at 8,000 rpm for 25 minutes at $0^0$C. The supernatant was collected in Teflon tubes and immediately used for the biochemical analysis in all experimental groups including control. Standard procedures used in clinical biochemistry laboratories based on protocol of commercial kits were followed for the determination of all biochemical parameters.

### Biochemical studies:

The total Protein content of the BPA exposed tissue samples was estimated according to modified standard method by (Lowery *et al*., 1951). Plasma glucose was determined using the Folin Malmros micro procedure as modified by (Murrell, L.R. and Nace, P.F., 1958).The activities of Aspartate aminotransferase (E.C.2.6.1.1) and Alanine aminotransferase (E.C.2.6.1.2) were assayed in different tissue homogenates by using the commercial kit provided by Asritha Diatech India Private Limited, Kukatpally, Hyderabad, Telangana, India. Plasma Creatinine value was determined according to Rock et al. (1987).

### Histopathology:

Both hepatic and renal tissue was fixed in Bouin's fluid for 12h. Afterwards both tissues were processed in graded ethanol series, cleared in xylene and finally embedded in paraffin wax (melting point $60^o$C). The embedded block were sectioned at 6µ on a rotary microtome, mounted on glass slides, dried and stained with haematoxylin and eosin (H&E). Sections were examined under a light microscope (NIKON ECLIPSE E 400, USA) and photographed by using digital camera attached to the microscope. Ten sections were analyzed per animal.

  

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**Ethical Statement**:
All experimentations were carried out in agreement with the guidelines of the Committee for the purpose of control and supervision of experiments on Animals (CPCSEA) (http://cpcsea.nic.in, Ministry of Environment, Forest and climate change, New Delhi).

**Statistical analysis**:
The collected data was analyzed statistically by implementing different statistical methods. Standard deviation and standard error was calculated by using MSXLSTAT software. The student's' test was performed to identify the levels of significance using statistical software XLSTAT version 2018.3. All the values of P below 5% level are labelled as significant, and the values above 5% level are chosen as non-significant. To validates the results; each assay was performed in triplicate.

## RESULTS

**Biochemical parameters:**
The results are summarized in table.1 Exposure to sublethal concentrations of BPA showed significant reduction in protein in both liver and kidney in all experimental groups while plasma glucose levels increased compared to healthy individuals. Conversely, the values of both *AST* (*SGOT), ALT* (*SGPT*) clearly shown a gradual and significant increase in all experimental fish treated with BPA. Plasma Creatinine values were ranged from 0.16mg/dl to 0.26mg/dl. Creatinine values were elevated in all experimental groups gradually in dose dependent manner.

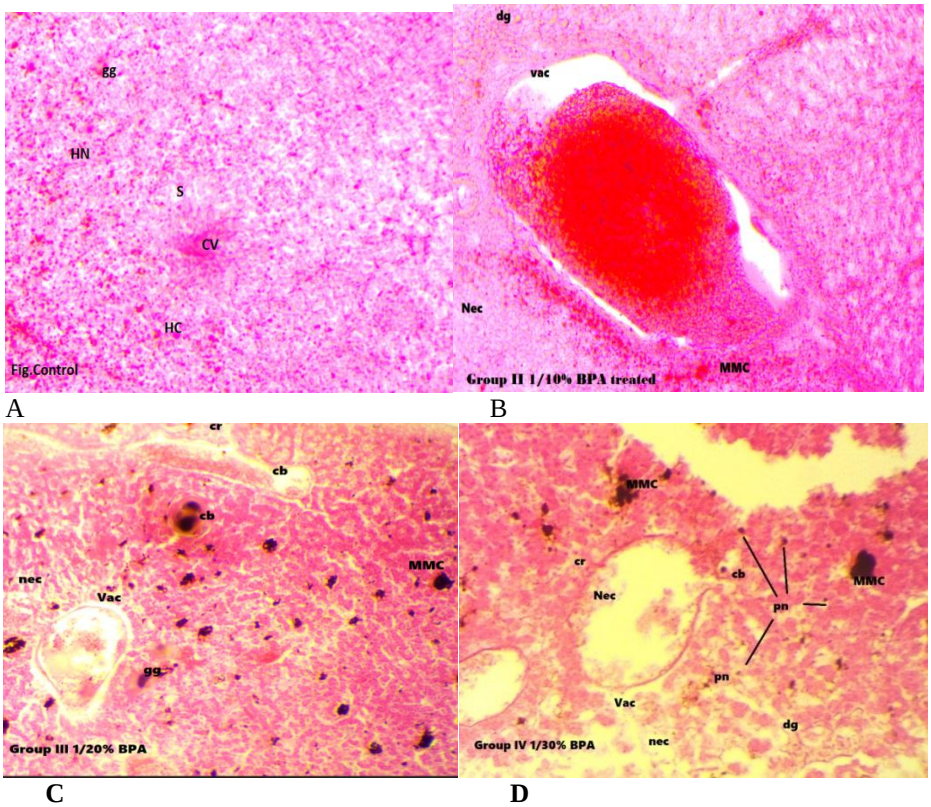


**PLATE 1.** Effects of sub lethal concentration of BPA on histopathology of liver of *Heteropneustes fossilis*. A. Control fish show normal structure. B.liver from Group II showing swelling. (x 1000) C. Histology of liver of *Heteropneustes fossilis* from Group III. Showing acute cellular swelling, hypertrophy and pyknosis .D. Showing acute cellular swelling, hypertrophy and pyknosis).Secondary infections in between hepatocytes are also seen. (All sections are H&E Stained x400).

  

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

| Group | | Total protein (µg/ml) | ALT (SGPT)(iu/l) | AST (SGOT) iu/l | Plasma Glucose (mg/dl) | Plasma Creatinine (mg/dl) |
|---|---|---|---|---|---|---|
| **Control** | Liver | 39.8± 0.31 | 42.2±2.11 | 68.2±2.11 | 29.8± 0.31 | 0.16± 0.004 |
| | Kidney | 8.6± 0.11 | 48.1± 1.8 | 48.1± 1.8 | | |
| **Group II** | Liver | 44.8± 0.41 | 56.8±2.87 | 146.8±2.87 | 34.8± 0.41 | 0.19± 0.005 |
| | Kidney | 9.8± 0.14 | 52.3± 2.2 | 52.3± 2.2 | | |
| **Group III** | Liver | 54.2 ± 0.51 | 94.5±3.69 | 134.5±3.69 | 44.2 ± 0.51 | 0.22± 0.003 |
| | Kidney | 18.8± 0.15 | 58.31± 2.7 | 58.31± 2.7 | | |
| **Group IV** | Liver | 64.6± 1.75 | 113.5±4.56 | 153.5±4.56 | 59.6± 1.75 | 0.26± 0.006 |
| | Kidney | 20.3± 0.13 | 62.41± 3.2 | 62.41± 3.2 | | |

**Histopathology of liver:**

Tissue alterations in the control fish were minimum, while the lesions found in fish from different experimental groups were more severe and in some cases irreparable reflecting the toxic effects of BPA. The results of histological alterations in the liver of control and experimental fishes are presented in figures Plate I. Fish liver from control group showed normal architecture with central vein (CV), sinusoid (S), hepatocytes (HC), hepatocyte nucleus (HN) and glycogen granules (gg). The hepatocytes of control liver were normal in appearance, with central nuclei, arranged in cords around central vein. However, the treatment of sublethal concentrations of BPA induced significant and severe histological anomalies like (ag) aggregation of melanomacrophages, (S) sinusoid, (BV) blood vessel (V) central vein, (nec) necrosis, (vac) vacuolation, (dg) patchy degeneration, (cb) blood congestion,(pn) pyknotic nucleus,(cr) cellular rupture, (gg) glycogen granules, and nuclear degeneration in dose dependent manner.

**Histopathology of kidney:**

The results of histological alterations in the kidney of control and experimental fishes are presented in figures PLATE I.

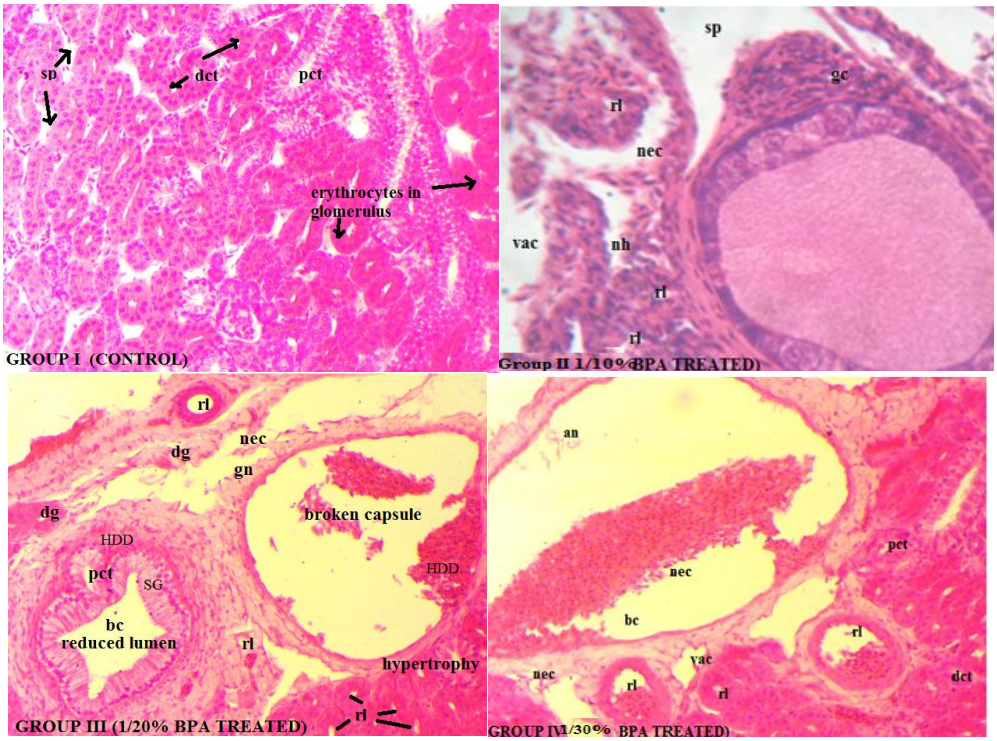


**Plate II. FIG. 25.**Effects of sub lethal concentration of BPA on histopathology of kidney of *Heteropneustes fossilis*. A. Control fish show normal structure. Kidney from Group II showing necrosis, nuclear hypertrophy, reduced lumen, vacuolation and spaces. (x 400) C. Histology of kidney from Group III showing cellular degeneration, necrosis, broken capsule and pyknosis .D. Histology of kidney from Group Iv showing reduced lumen, tubular degeneration, decreased the tubular lumen, hypertrophy and pyknosis. (All sections are H&E Stained x400).

  

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Photomicrograph of *H.fossilis* kidney from control group showed normal slightly spherical glomeruli with proper bowman space. Brush border of proximal tubules (pct) and lumen of distal tubules (dct) showed normal structure (Plate II).In fish treated with various sub lethal concentrations of BPA shown several anomalies such as (ag) aggregation of melanomacrophages (nec) necrosis (vac) vacuolation, (dg) degeneration (cb) blood congestion (pn) pyknotic nucleus (cr) cellular rupture, nuclear hypertrophy (nh) degeneration (dg) reduction of lumens (rl).

**DISCUSSION**

In general, blood biochemical values are not usually employed as analytical tool in fish due to lack of reference standards plus changes in blood analysis in fish are linked with specific diseases and metabolic disorders that are not well understood. The present investigation is mainly concerned with comparative evaluation of enzymatic activities and histopathological anomalies after BPA intoxication.

The liver plays a central role in degradation and detoxification of pollutants. Alanine aminotransferase (ALT) plays a key role in synthesis and deamination of amino acids during stress imposed situations to accomplish high-energy demand of the organism (Samanta *et al,* 2014). In the present study, ALT activity after exposition of sublethal concentration of BPA under laboratory condition was significantly increased ($P< 0.05$) in both the tissues when compared to control value (Table 1). The results of present study clearly shown that ALT activity was elevated highly in liver in comparison of kidney. This increased activity is seems to be tissue specific. The higher levels of ALT in both tissues of the experimental fishes clearly indicated tissue damage, which perhaps due to disruption in normal physiological, and biochemical processes such as Krebs' cycle, and TCA cycle. Consequently, leakage of this enzyme was happened from the liver cytosol through membrane into the blood stream (Samanta *et al,* 2014).The present results were in agreement with the results of Vutukuru, S.S., *et al* (2007) who detected increased activity of serum ALT in in Indian major carp, *Labeo rohita*, exposed to arsenic and chromium. Chaudhari *et al* (2017) found elevated levels of serum ALT activity in *Cirrhinus mrigala* exposed to thermal power plant effluent (TPPE). Recently, Dar, S.A., *et al* (2018) also observed increased activity of ALT in *Labeo rohita* treated with benzimidazole derivatives. Another recent study of Kumar, N., *et al* (2018) also confirmed the higher levels of ALT activity in fish *Pangasius hypophthalmus treated with* selenium (Se) and selenium nanoparticles. The higher activity of ALT in BPA exposed fish might provide the oxaloacetic acid and pyruvate to meet the increased energy demand during BPA induced stress condition as in the fish *Clarias batrachus* exposed to Carbofuran (Begum, 2004).

Aspartate aminotransferase (AST), even though a specific enzyme of liver but also found in high amounts in skeletal muscle cells and kidney. It supports gluconeogenesis from amino acids in association with ALT. In the present study, the AST activity was also increased significantly ($P< 0.05$) when compared to control value in both the tissues. Higher AST activity in the experimental fish tissues under BPA sublethal treatment was a warning signal of damage either in tissues or in organs leading to discharge of the enzyme into the blood circulation. Another reason for elevated levels of these enzymes may be due to hepatic cells injury or increased synthesis of the enzymes (Rastiannasab, A., *et al,* 2016). Various authors in different fish tissues exposed to various pollutants also reported similar results of increased AST activity (Begum, 2004, Rastiannasab *et al,* 2016, Chaudhari *et al,* 2017; Dar *et al,* 2018; Ranjan *et al,* 2018).

In the present study, the total protein content of both liver and kidney significantly decreased in fishes exposed to different sublethal concentrations of BPA compared to control. The reduced values of total protein particularly in liver may be due to depletion in tissue proteins or due to impaired or low rate of protein synthesis under BPA induced stress or due to their utilization in the formation of mucoproteins. Besides, direct and / or indirect application of proteins for energy requirements and cellular repair may be a reason for total protein depletion (Vutukuru, 2005).

Glucose is the major source of energy in all vertebrates including fish and is a sensitive indicator of environmental stress in fish (Silbergeld, 1974). Fish blood comprises, normally, 40-90 mg/dl of glucose (Patriche, 2009). Plasma glucose in the present study displayed significant elevated values of glucose ($P > 0.05$) (hyperglycemia) in all three exposed groups of fish compared to control. The higher values in blood glucose concentrations are measured as a reliable indicator of environmental stress believed as a general secondary response to stress induced by toxicants like BPA (Fırat *et al.* 2011). Under stressful environments, an organism including fish practices energy reserves in the form of glycogen in the muscles and liver that in turn increases glucose concentration in plasma (Bartoňková, J., *et al* 2017). This increase may be due to glycolysis in response to stress for the demand of more energy requirement by fish. The

  

hyperglycaemic response shown in the present investigation is a sign of a disruption in carbohydrate metabolism, probably due to enhanced hepatic glucose 6-phosphatase activity, that elevated breakdown of glycogen or the synthesis of glucose from other tissue proteins and amino acids (Fırat *et al*, 2011). However, stress exposure affects several physiological and immunological indices in fish. Jentoft *et al* (2005), recorded increased level of plasma glucose concentrations in Eurasian perch (*Perca fluviatilis*) and domesticated rainbow trout exposed to the standard handling stressor. The hyperglycemia in BPA exposed fishes possibly resulting from activation of the pituitary inter renal axis by the stress of BPA causing mobilization of tissue glycogen.

In the current investigation, serum creatinine levels were elevated significantly in *H.fossilis* administered with sublethal concentrations of BPA compared with control group. The enhanced levels of serum creatinine may be due to excessive damage of tissue or dietary or weaken excretion, increased synthesis, or reduced degradation activity of BPA (Amin and Hashem, 2012). The present results confirm that BPA treated fish suffered with glomerular dysfunction as blood levels of creatinine depend largely on glomerular function. Our results are in agreement with Amin, K.A. and Hashem (2012), in catfish *Clarias gariepinus* administered with deltamethrin and Solomon and Oguike, (2017), in *Clarias gariepinus* and *Tilapia niloticus* from commercial fishponds.

The use of histopathological studies is endorsed as more reliable valuations of biochemical responses in fish exposed to a variety of environmental contaminants (Reddy and Rawat, 2013; Reddy and Kusum, 2013; Kumar *et al.*, 2018). In the present experiment, a range of histopathological alterations was found in both liver and kidney. However, the severity and occurrence of lesions was found to be more noticeable in fish exposed to higher concentrations of BPA. Liver tissue of control fish was characterized by the presence of a high amount of glycogen within irregular shaped vacuoles. However, a clear reduction of glycogen deposits was seen within the hepatocytes when fish exposed to various sub lethal concentrations of BPA. Furthermore, aggregation of melanomacrophages (ag), sinusoid, (S) necrosis (nec), vacuolation (vac) patchy degeneration (dg), blood congestion (cb) pyknotic nucleus (pn), and cellular rupture (cr) were also noticed in liver of experimental fish).

In the present study, the kidney of control fish exhibited a normal architecture and there were no pathological abnormalities. However, sublethal concentrations of BPA treatment produced many severe alterations including (ge)glomerular expansion, (rb) reduction of Bowman's space (ag) aggregation of melanomacrophages (nec) necrosis (vac) vacuolation, (dg) degeneration (cb) blood congestion (pn) pyknotic nucleus (cr) cellular rupture (nh) nuclear hypertrophy (dg) degeneration reduction of lumens (rl)in the kidney. The key mechanism involved in histopathological changes in the present study is that BPA exerted its toxic properties by increasing the oxidative stress, which in turn induced various histological anomalies in the concerned tissue.

The increased oxidative stress caused elevated lipid peroxidation on cell membrane, which might cause degenerative changes in tissue (Faheem *et al* 2016, Kothari, S. and Parihar, M.S 2017, Patil A and Reddy, P.B. 2017, Huggett, R.J., 2018). The histopathological and biochemical changes observed in the present study are indicative of severe hepatic and renal dysfunction in H.fossilis exposed to sublethal concentrations of BPA.

## CONCLUSIONS

The present investigation validates the alterations in biochemical enzyme activities of total protein, plasma glucose, creatinine, ALT, and AST, in the liver and kidney of fish under the sub lethal concentrations of BPA treatment, which finally affected the fish health.

The maximum severe alteration in enzyme activities were observed in liver as liver plays prime role in detoxification of the toxicants. Overall, our results advocate that the selected biochemical and histopathological parameters are valuable indicators for the assessment of BPA and other compound impacts in natural aquatic environments. Further studies are required for investigating the oxidative stress mechanism at molecular level involved in this process.

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