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Bisphenol S Induced Metabolic Disruption in a Freshwater Fish, *Oreochromis Mossambicus*

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Abstract

Bisphenol S (BPS) is an analogue of Bisphenol A (BPA), a legendary xenoestrogen used to produce polycarbonates, thermal receipt papers and epoxy resins. The present study evaluated the effects of sublethal concentrations of BPS (100, 125 and 150mg/ l BPS) in *Oreochromis mossambicus* (45 ± 2g, 8 ±1cm) exposed to a period of 4, 8 and 12 days. The study investigated the effects of sublethal concentrations of BPS on enzymes involved in mitochondrial metabolism such as mitochondrial malate dehydrogenase (MDH), nicotinamide adenine dinucleotide (NADH) dehydrogenase, succinate dehydrogenase (SDH) and cytochrome c oxidase (CCO) in the hepatic tissue of *O. mossambicus*. The results revealed that exposure of BPS significantly influenced the activities of the mitochondrial enzymes in fish. Changes in the activities of the above said mitochondrial enzymes in fish might affect Krebs cycle and thereby energy production. The present study also analysed the changes in the activity of enzymes involved in intermediary metabolic pathways such as glucose 6 phosphatase ($G6PO_4$ase), malic enzyme (ME), isocitrate dehydrogenase (ICDH) and lactate dehydrogenase (LDH). Significant variations ($P<0.05$) occurred in the activities of intermediary enzymes, which may disrupt metabolic pathways. This study assumes significance in determining the effect of BPS as a metabolic disruptor. In conclusion, BPS impairs the activity of intermediary and mitochondrial metabolic enzymes and thereby affects the enzymes of tricarboxylic acid cycle and electron transport system.

Keywords: Xenoestrogen, *Oreochromis mossambicus*, Sublethal, Krebs cycle, Electron transport system

1. Introduction

Bisphenols are a group of chemicals which are used to manufacture plastics, epoxy resins and other products since the 1960s. Bisphenol A (BPA) is the most commonly used bisphenol used to produce plastic containers, adhesives, paints, dental sealants and household products (Yang *et al.*, 2015). Its use in consumer products was prohibited due to its properties as an endocrine disruptor in 2015 (European Commission, 2011). Exposure of BPA is a concern because of its possible health effects on different tissues and organs, including the reproductive system, immune system and neuroendocrine system in the human population (Sheehan *et al.*, 1999), and as a result, several bisphenol analogues like bisphenol B, bisphenol F and bisphenol S have been introduced as a replacement in various applications. Bisphenol S (BPS) is used as a substitute for the potentially toxic BPA, without knowing its possible harmful effects like cancer, metabolic disorders, obesity, DNA damage, liver damage, endocrine and reproductive effects (Kuruto- Niwa *et al.*, 2005, Lee *et al.*, 2013). It has two phenol functional groups attached, one on each side to the central sulphur atom of a sulfonyl group. It has increased stability against high-temperature range and is more resistant to sunlight; hence, less biodegradation in the environment (Pivnenko, 2015). The products labelled 'BPA free' are gradually replaced by BPS (Grignard *et al.*, 2012 and are now used in manufacturing plastics, thermal receipt papers, currency bills and food cartons (Liao *et al.*, 2012, Pivnenko, 2015).

Experiments have proven that BPS also acts as a kind of endocrine disruptors (EDs) (Kuruto-Niwa et *al.*, 2000; Cao *et al.*, 2013) and presents a potential threat to human health. Contrary to BPA, there is not much research done regarding the effects of BPS in organisms. (Wu *et al.*, 2018). However, since the use of BPS in the industry is increasing, some studies have been done: Chronic exposure of BPS to zebrafish larvae induces developmental deformities in several organs, including the heart (Moreman et *al.*, 2017, Mu *et al.*, 2018). Ji *et al.* (2013) showed that exposure to low levels of BPS could affect the feedback regulatory circuits of the hypothalamo-pituitary-gonadal axis and impair offspring development in zebrafish (*Danio rerio*).

Fish are used to determine aquatic systems' health because their biological responses serve as biological markers of environmental pollution (Linde-Arias *et al.*, 2008). Studies on metabolic disruption are not given due recognition in toxicological research. Nonetheless, these studies have an essential role in the closer investigation of the physiological activities of organisms. Moreover, activities of tricarboxylic acid (TCA) cycle enzymes are used as indices in assessing regular metabolic rate, stress and aerobic activity in fish on exposure to xenobiotics (Dahlhoff, 2004). The enzymes investigated in intermediary metabolism in the present study are glucose 6 phosphatase ($G6PO_4$), malic enzyme (ME), isocitrate dehydrogenase (ICDH) and lactate dehydrogenase (LDH) and the enzymes scrutinized in the mitochondrial metabolism are mitochondrial malate dehydrogenase (MDH), nicotinamide adenine dinucleotide (NADH) dehydrogenase, succinate dehydrogenase (SDH) and cytochrome c oxidase (CCO). Since the liver plays a

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remarkable role in detoxification of metabolites, protein synthesis, and biochemical production necessary for digestion and growth (Maton *et al.*, 1993, Chiang, 2014) in vertebrates, the current study analyzed the changes in intermediary and mitochondrial pathways in the liver of freshwater fish, *Oreochromis mossambicus*, if any, induced by sublethal dosages of BPS.

2. Materials and Methods

2.1. Fish and Aquaria

The animal model used in the experiment is a freshwater fish, *O. mossambicus*, of body weight 45 ± 2g and length 8 ±1cm. Fish were collected from a fish farm in Thiruvananthapuram and brought to the laboratory. Before the experiment, the fish were acclimatized for 30 days in large cement tanks (200L) filled with dechlorinated tap water. Water temperature (28 ± 2°C), oxygen saturation (70-100%) and pH (7.6) were monitored using standard procedures. Fish were fed daily on protein feed *ad libitum*.

2.2. Test chemical

The test chemical used in the experiment was BPS of 98% purity, purchased from Sigma-Aldrich Bengaluru, India. The LC_{50} value in 96hr time interval was determined by probit analysis [Organisation for Economic Co-operation and Development (OECD) guidelines], with a confident limit of 5 % level (Finney, 1971). The lethal concentration for 50 % killing (LC_{50}) values was computed for 96hr exposure and was found to be 200mg/l BPS for fish. Based on that, three sublethal doses such as 100, 125 and 150mg/l BPS were selected for treatment. The mortality and the behaviour of fishes in each group were monitored throughout the experiment at varying concentrations.

2.3. Experimental design

Before the experiment, fish were divided into 10 groups of six each in separate well-aerated aquarium glass tanks (40 L) of dimension 31 x 41 x 53cm**.** The fish were exposed to different concentrations of BPS with five replicates for each concentration, where maximum 6 fish were used per concentration of the test toxicant. The freshwater sample having the specified doses of BPS was replaced on alternate days. Group I fish was maintained as control and kept in dechlorinated tap water. Fish in groups II to X were exposed to 100mg/l, 125mg/l and 150mg/l BPS for a period of 4, 8 and 12 days, respectively.

2.4. Sampling

The fish were starved for 24hr before sacrifice for getting optimum experimental conditions. After stipulated periods of exposure, fish were killed, and 100- 200mg liver of each fish were immediately dissected and frozen at -20^{0}C in the deep freezer for biochemical assays.

2.5. Assay of intermediary metabolic enzymes

Chilled liver was separately homogenized at 4^{0}C in 0.25ml SET buffer (pH 7.4) [0.25M sucrose solution containing 1mM EDTA (Ethylenediaminetetraacetic acid), 20mM tris HCl (pH 7.02) and 0.2% Bovine serum albumen (BSA). Homogenization was done at 2000rpm, 4^{0}C for 10min and the supernatant was used for the assay.

i.Glucose 6 Phosphatase ($G6PO_4$ase; D-Glucose-6-Phosphatase Phosphohydrolase., EC.3.1.3.9)

Glucose 6 Phosphatase activity was estimated by the method of Swanson (1955). An assay mixture containing 0.3ml of citrate buffer (pH 6.5) and 0.2ml of glucose-6-phosphate (pH 6.5) were pre-incubated in separate test tubes labelled test and control for 5min. Tissue extract (0.1ml) was added to the test after mixing the content; the test and control were incubated for 15min at room temperature. After incubation, 1ml of 10% TCA (Trichloroacetic acid) was added to both tubes to arrest the reaction. Then tissue extract (0.1ml) was added to the control, mixed well, and the test and control were centrifuged for 10min at 3000rpm. For the assay, the supernatant collected was analyzed by the method of Fiske and Subbarow, (1925). The absorbance was monitored using a UV visible spectrophotometer at 640nm, and the results were expressed as nanomoles inorganic phosphate liberated /min/mg protein.

ii. Lactate dehydrogenase (LDH., E.C.1.1.27)

The activity of LDH was measured by the method described by King (1965). To 0.1ml of homogenate in the test tubes, 0.1ml of NADH solution was added to each test tube. The tubes were pre-incubated in a water bath at 37^{0}C for about 3min. The reaction was started by adding 1ml sodium pyruvate solution to each test tube in sequence at timed intervals. The solutions were well mixed and incubated for 30min. After exactly 30min, the reaction was stopped by adding 1ml of 2, 4-dinitrophenylhydrazine. Removed the test tubes from the water bath; it was mixed and allowed to stand at room temperature for 20min. After 20min, 10ml of NaOH (Sodium hydroxide) was added to each test tube and allowed it to stand for 5 to 10min and then read the absorbance at 550nm setting the spectrophotometer to zero with distilled water. Results were expressed as IU/min/mg protein.

iii. Malic Enzyme (ME; $_{L-}$malate: $NADP^+$ Oxidoreductase., EC.1.1.1.40)

The activity of ME was determined spectrophotometrically at 340nm according to the method specified by Ochoa, (1955a). The assay mixture consisted of 300ml glycylglycine buffer, 100ml $MnCl_2$ and 200ml of L- malate. Then the volume was made up to 3ml by adding double-distilled water. To the reaction mixture, 100µl of tissue homogenate was added, and the addition of NADP+ started the chemical reaction. The specific activity was represented as 1U/min/mg protein.

iv. Isocitrate dehydrogenase (ICDH; Isocitrate dehydrogenase $NADP^+$., E.C.1.1.1.42)

The activity of ICDH was analyzed by the method of Ochoa, (1955b). The reaction mixture consisted of 0.3ml glycylglycine buffer, 0.1ml $MnCl_2$ and 0.2ml isocitrate. The aliquot was made up to 2.8ml by adding double distilled water. 100µl tissue extract was added, and then the reaction was initiated by the addition of $NADP^+$. The change in OD was measured at 340nm for 2min against a blank containing all components except tissue homogenate. The enzyme activity was expressed as IU/min/mg protein.

2.6. Isolation of mitochondria and assay of enzymes

Mitochondria were isolated from the liver of fish by using the method depicted by Irving and Watson, (1976), and

all the mitochondrial enzymes were assayed spectrophotometrically (Perkin Elmer, USA) at room temperature.

i. Malate dehydrogenase (MDH; $_{L\ Malate}$: NAD^+Oxidoreductase., EC.1.1.1.37)

Malate dehydrogenase activity in hepatic tissue was analyzed by the method of Mehler *et al.* (1948). The reaction mixture consisted of 300µl glycylglycine buffer and 25µl of oxaloacetate. The volume was adjusted to 2.8ml by adding double-distilled water. 100µl of tissue homogenate was added to the assay mixture. Then the reaction was started by adding NADH. The enzyme activity was recorded at 340nm, and specific activity expressed as IU/min/mg protein.

ii. NADH dehydrogenase., (E.C.1.6.5.11)

NADH dehydrogenase activity was estimated by using the method of Minakami *et al*. (1962). The reaction mixture contained 1.0ml of phosphate buffer, 0.1ml of Potassium ferricyanide, 0.1ml of NADH and 0.2ml of mitochondrial suspension. The total volume was made up to 3ml with double distilled water. NADH was added just before the addition of the enzyme. A control was also treated similarly without NADH. The change in OD was measured at 420nm as a function of time for 3min at an interval of 15sec in a UV visible spectrophotometer. The activity of NADH dehydrogenase was expressed as moles of NADH oxidized/min/mg protein.

iii. Succinate dehydrogenase (SDH; Succinate ubiquinone oxidoreductase., E.C.1.3.5.1)

The activity of SDH was assayed according to the method of Slater and Bonner (1952). The reaction mixture containing 1ml phosphate buffer, 0.1ml of EDTA, 0.1ml of BSA, 0.3ml of sodium succinate and 0.2ml of potassium ferricyanide was made upto 2.8ml with double distilled water. To the assay mixture 0.2ml of mitochondrial suspension was added. The change in OD (optical density) was recorded at an interval of 15 sec for 5 min at 420nm. The SDH activity was expressed as nmoles of succinate oxidized/min/mg protein.

iv. Cytochrome c oxidase (CCO., E.C.1.9.3.1)

The activity of CCO was measured according to the method of Pearl *et al*. (1963). The reaction mixture contained 1.0ml of phosphate buffer, 0.2ml of N-phenyl-P-phenylene diamine, 0.1ml of 0.01% cytochrome c and 0.5ml of water. The sample was incubated at 25°C for 5min. 0.2ml of the enzyme preparation was added, and the change in OD was recorded at 550nm for 5min at an interval of 15sec each in a UV visible spectrophotometer. A control containing all reagents except cytochrome c also proceeded in the same manner. The activity of cytochrome c oxidase was expressed as a change in IU/min/mg protein.

2.7. Estimation of Protein

Protein concentration was measured according to the protocol of Bradford (1976).

2.8. Statistics

Data collected was analyzed by ANOVA. The difference in means was tested by Duncan analysis (1955) using SPSS software. $P < 0.05$ was considered as significant level.

3. Results

3.1. The activity of Intermediary Enzymes

3.1.1. Glucose 6 Phosphatase

The effect of $G6PO_4$ in fish exposed to BPS for 4, 8 and 12 days is shown in Fig.1. There was a considerable increase in $G6PO_4$ activity after 4 days of exposure to 100 mg/l, but a slight decrease was observed on 8th day, and then an addition was shown after 12 days of exposure when compared to control. A significant decrement was noted on exposure to sublethal doses (125 and 150 mg/l) of BPS after 4, 8 and 12 days.

3.1.2. Lactate Dehydrogenase

The activity of LDH is depicted in Fig.2. LDH activity depleted significantly when exposed to sublethal doses (100,125 and 150 mg/l) of BPS after 4, 8 and 12 days of exposure.

3.1.3. Malic Enzyme

Fish, when exposed to 100mg/l BPS showed an initial drastic increase in ME activity after 4 days of exposure. The ME activity declined after 8 and 12 days but was still significantly higher concerning control (Fig.3). Exposure to 125 and 150 mg/l BPS resulted in a slight increase in ME activity after 4 and 8 days of exposure, while a significant sharp increase was observed after 12 days of exposure.

3.1.4. Isocitrate Dehydrogenase

There was a significant fall in ICDH activity in hepatic tissue when exposed to sublethal doses (100,125 and 150 mg/l) of BPS after all days of exposure when compared with the control (Fig.4)

3.2. The activity of Mitochondrial Enzymes

3.2.1. Malate Dehydrogenase

Malate dehydrogenase activity depleted significantly in hepatic tissue of fish exposed to sublethal doses (100,125 and 150 mg/l) of BPS after 4, 8 and 12 days when compared to control (Fig.5)

3.2.2. NADH Dehydrogenase

The activity of NADH dehydrogenase on exposure to BPS for 4, 8 and 12 days is indicated in Fig 6. There showed a significant decline in NADH activity on exposure to sublethal doses (100,125 and 150 mg/l) of BPS after 4, 8 and 12 days.

3.2.3. Succinate Dehydrogenase

The activity of SDH in the hepatic tissue of fish exposed to BPS is depicted in Fig 7. There was a significant depletion in SDH activity after 4, 8 and 12 days of exposure to 100,125 and 150mg/l BPS.

3.2.4. Cytochrome c Oxidase

The activity of CCO in fish exposed to BPS is shown in Fig. 8. Initially, the activity was found to increase after 4 days of exposure to 100 and 125mg/l of BPS. There was a rapid decrease in CCO activity after 8 and 12days of exposure to 100 and 125 mg/l of BPS. Fish, when exposed to 150mg/l BPS showed a significant reduction in CCO activity after 4, 8 and 12 days.

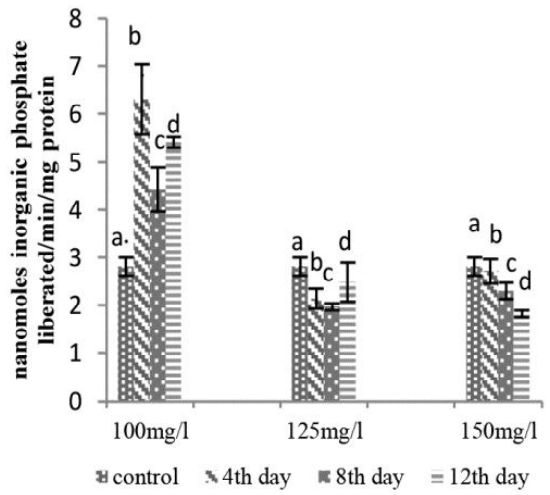

Fig. 1. The effect of Sub-lethal doses of BPS (100,125 and 150mg/l) on the activity of $G6PO_4$ase in the liver of *O.mossambicus.*

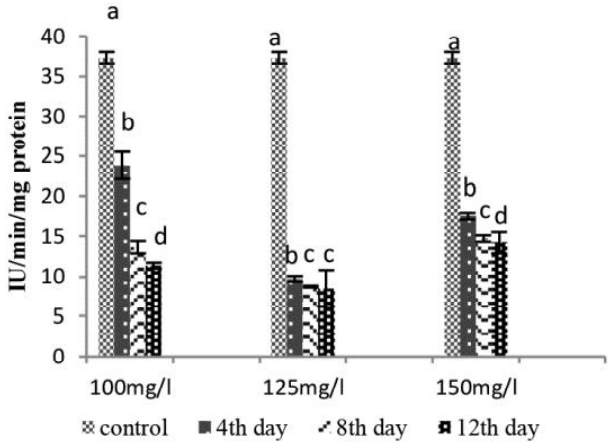

Fig. 2. The effect of Sub-lethal doses of BPS (100,125 and 150mg/l) on the activity of LDH in the liver of *O.mossambicus.*

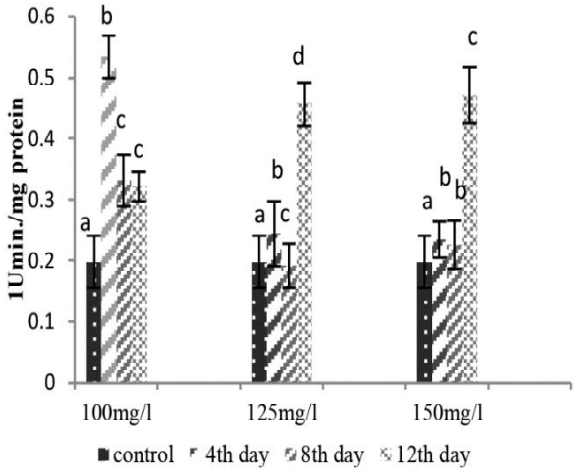

Fig. 3. The effect of Sublethal doses of BPS (100,125 and 150mg/l) on the activity of ME in the liver of *O.mossambicus.*

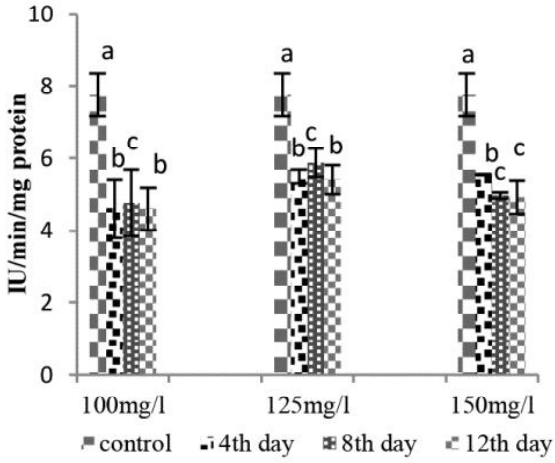

Fig. 4. The effect of Sublethal doses of BPS (100,125 and 150 mg/l) on the activity of ICDH in the liver of *O.mossambicus.*

The effect of sublethal doses of BPS (100,125 and 150mg/l) on the activities of $G6PO_4$ase (Fig.1), LDH (Fig.2), ME (Fig. 3) and ICDH (Fig.4) in the liver of *O. mossambicus* in relation to control. Each bar is mean ± SEM for six fish. Means with different superscript letters (a, b, c and d) for each parameter is significantly different at $P<0.05$.

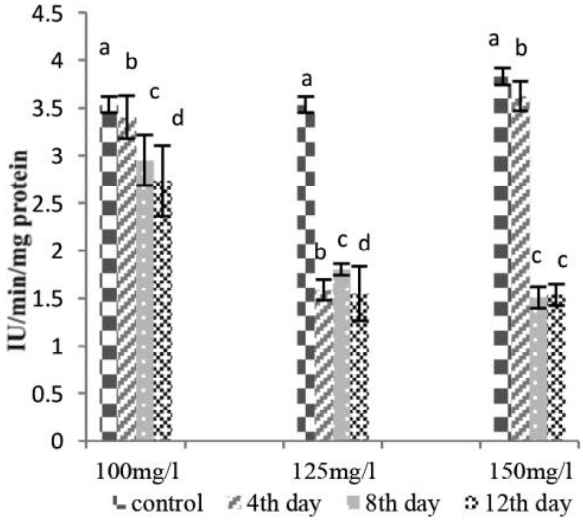

Fig. 5. The effect of Sublethal doses of BPS (100,125 and 150 mg/l) on the activity of MDH in the liver of *O.mossambicus.*

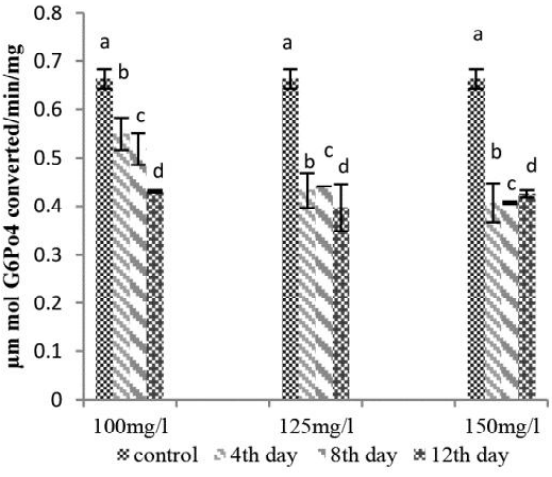

Fig. 6. The effect of Sublethal doses of BPS (100,125 and 150 mg/l) on the activity of NADH dehydrogenase in the liver of *O.mossambicus.*

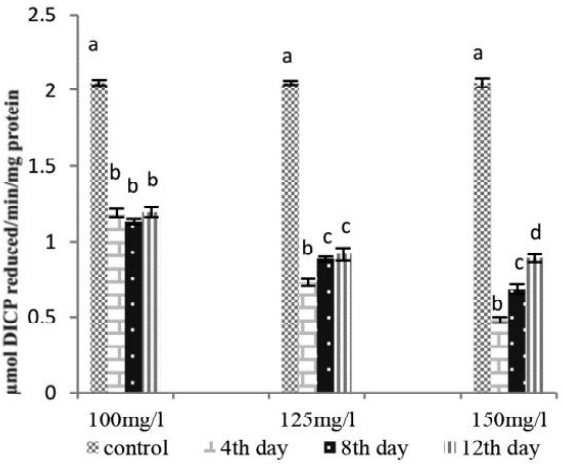

Fig. 7. The effect of Sublethal doses of BPS (100,125 and 150 mg/l) on the activity of SDH in the liver of *O.mossambicus.*

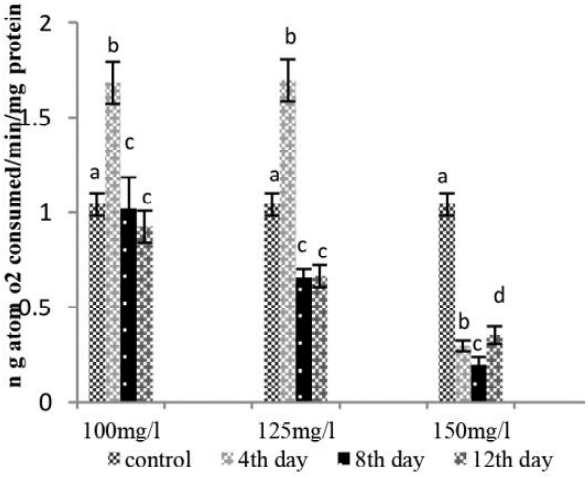

Fig. 8. The effect of Sublethal doses of BPS (100,125 and 150 mg/l) on the activity of CCO in the liver of *O.mossambicus.*

The effect of sublethal doses of BPS (100,125 and 150mg/l) on the activities of MDH (Fig.5), NADH dehydrogenase (Fig.6), SDH (Fig.7) and CCO (Fig.8) in the liver of *O. mossambicus* in relation to control. Each bar is mean ± SEM for six fish. Means with different superscript letters (a, b, c and d) for each parameter is significantly different at P<0.05.

4. Discussion

The present investigation showed a decrease in the activity of $G6PO_4$ase in fish. Glucose 6 phosphatase is the key enzyme in glucose homeostasis, functioning in both the processes of gluconeogenesis and glycogenolysis. This enzyme is an integral membrane protein found in association with the lumen of the endoplasmic reticulum (Herman and Nordlie, 1972). It hydrolyzes glucose 6 phosphates to glucose in the endoplasmic reticulum. A decrease in $G6PO_4$ase activity was observed in freshwater teleost, *Mystus vittatus* after chronic exposure to metasystox and sevin (John, 2007) and in *Aplochelius lineatus* exposed to glyphosate (Anjali and Aruna Devi, 2016). In fish, inhibition of $G6PO_4$ase activity may be due to the damage to microsomal membrane as this enzyme is localized exclusively in the membranes of the endoplasmic reticulum (Alexander, 2013). Gluconeogenic progression was affected by the decreased activity of $G6PO_4$ase which simultaneously restricts glucose formation (Zhang *et al*., 2019).

Lactate dehydrogenase activity was found to be significantly declined in fish exposed to BPS. The LDH is an anaerobic enzyme involved in the conversion of pyruvate to lactate in glycolysis. It is an important glycolytic enzyme in biological systems and is inducible by oxygen stress. Therefore, the activity of several regulatory enzymes may be significantly changed to meet the required energy demands under toxic stress (Mayer *et al*., 1989), including the activity of lactate dehydrogenase (LDH), which helps the continued process of glycolysis under anaerobic conditions (Diamantino *et al*., 2001). Almeida *et al*. (2001) reported the inhibition of LDH activity in the freshwater murrel, *Channa punctatus* exposed to mercuric chloride and in Nile tilapia, *Oreochromis niloticus* exposed to cadmium; which might be due to changes in the activity of mitochondrial membrane function. Elumalai *et al.* (2001) noted a reduced activity of LDH in the crab, *Carcinus moenas* maintained in water contaminated with copper, chromium or a mixture of both. The decrease in NAD dependent LDH activity also indicates the accumulation of lactic acid (Diamantino *et al*., 2001). Henceforth, LDH can be used to evaluate the metabolic toxicity of BPS in fish.

In the current investigation, ICDH activity was found to be decreased in *O. mossambicus.* Isocitrate dehydrogenase is structurally bound to mitochondria which is an important enzyme due to its function in the aerobic pathway of energy production (Napierska and Podolska, 2006).

This enzyme catalyses the reversible oxidation of isocitrate to oxalosuccinic acid, followed by decarboxylation, leading to the formation of á-ketoglutarate. The control of mitochondrial redox balance and oxidative damage is one of the primary functions of NADP+-dependent isocitrate dehydrogenase. Ivanina *et al*. (2008) reported a decrease in ICDH activity in combined effects of cadmium and temperature on key mitochondrial enzymes, including NAD- and NADP-dependent ICDH in Crassostrea virginica in fry of common carp, *Cyprinus carpio* at different pH (Reddy *et al*., 2008), in juvenile larger yellow croaker *Larimichthys croceus* exposed to high copper intake (Meng *et al*., 2016) and in *Aplochelius lineatus* exposed to glyphosate (Anjali and Aruna Devi, 2016). The reduced activity of ICDH in the liver may influence energy and redox status and decrease the activation energy of mitochondrial metabolism in the experimental fish. Moreover, it might lead to a decrease in ATP synthesis due to oxidative phosphorylation uncoupling. Hence, BPS can be considered as uncouplers of oxidative phosphorylation.

Malic enzyme activity was found to be elevated significantly in fish when exposed to BPS. This enzyme catalyses the oxidative decarboxylation of malate to yield pyruvate in the presence of a divalent metal ion. The products of this reaction – pyruvate, CO_2 and NADPH – are used in a wide range of metabolic pathways as a source of carbon and reductive power. Malate functions as an intermediate in TCA cycle and is involved in malate –

aspartate shuttle. Malate- aspartate shuttle is an important mechanism used for the movement of reducing equivalents in the form of NADH from the cytoplasm to mitochondria for energy production. According to McKenna *et al*. (1995), the malic enzyme activity has an important role in the complete oxidation of intermediate compounds in TCA cycle for energy. In the current study, an increase in activity of malic enzyme oxidizes malate very rapidly, which may lead to rapid depletion of malate in cells and thereby affect redox potential.

From the present study, it can be visualized that there is a significant decrement of SDH activity in the liver of fish exposed to sublethal concentrations of BPS. Succinic dehydrogenase is an essential enzyme whose activity takes part in oxidation of carbohydrate through Krebs cycle (Singer *et al*., 1973). Being a key enzyme in the TCA cycle, it is assumed that with the inhibition of SDH activity, the metabolic path might have turned to an anaerobic pathway to meet the increased energy demands during stress. The decrease in this enzyme activity might be probably due to mitochondrial damage and decreased state of respiration (Bag *et al*., 1999). Since SDH is a mitochondrial enzyme, the level of its activity is an index of the mitochondrial as well as the oxidative metabolism occurring in the tissues concerned. The decrease in SDH activity may be due to the disorganization of mitochondria, affecting enzymes of TCA cycle.

The general decrease in SDH activity was associated with the inhibition of the mitochondrial respiratory mechanism. This prevents electron transfer to molecular oxygen, thereby inhibiting SDH activity which results in shifting of aerobic metabolism to the anaerobic pathway (Agrahari *et al.*, 2007). When *Tilapia mossambica* and *Clarias gariepinus* exposed to different type of chemicals, the activity of SDH found to be decreased due to depletion in the oxidative metabolism at the level of mitochondria leading to depression of TCA cycle (Sudharsan *et al*., 2000, Al Ghanim and Mahboob, 2012). Decreased activities of LDH and SDH were also observed in fish *Colisa fasciatus* due to toxicity of cypermethrin (Singh *et al.*, 2010). Decreased activity may be accounted for as the fish depends more on the anaerobic metabolic pathway for its energy needs under toxic stress. The suppression of SDH activity in *O.mossambicus* impairs the oxidative metabolic cycle and hence depends upon anaerobic glycolysis.

The current study observed a significant decrease in MDH activity in the liver during exposure periods of BPS. Malate dehydrogenase converts malate to oxaloacetate and also plays a significant role in CO_2 fixation and in gluconeogenesis. Since MDH is an indispensable enzyme in the oxidative metabolic pathway of Krebs cycle for carbohydrates, fats and amino acids, any interference in its activity will affect energy production in fish. Furthermore, MDH is a foremost enzyme in the Krebs cycle, and its decreased activity in the current study indicates interference in the Krebs cycle. Tiwari *et al*. (2009) observed the decreased MDH activities in tissues of *Clarias batrachus* on exposure to endosulfan. The decrement of MDH activity suggests that there is a shift in respiratory metabolism towards anaerobiosis. It also indicates that a decrease in the activity may lower the functioning of the Krebs cycle due to inadequate supply of substrate or decreased oxygen uptake at the tissue level during stress and may be due to inhibition of oxidative metabolism.

When experimental fish is exposed to sublethal doses of BPS, the activity of CCO decreased. Cytochrome c oxidase is the farthest enzyme in the respiratory electron transfer system (ETS) and helps transfer electrons to their final acceptor oxygen in ETS. It produces ATP molecules, thereby influencing other cellular metabolic processes. The activity of this enzyme apprises on the aerobic capacity of tissues. Sublethal experiment with cypermethrin causes a decrease in CCO activity in liver and muscle tissues in fingerlings of common edible carp (*Labeo rohita*), which shows a profound impact on oxidative metabolism (Tiwari *et al*., 2012). Exposure to glyphosate decreased the activity of CCO in *Aplochelius lineatus* (Anjali and Aruna Devi, 2016). The decrease in CCO activity might be due to the reduced availability of oxygen and thereby reduced the capacity of the ETS to produce ATP molecules (Corbett *et al.*, 1984). The decreased activity of CCO shows a clear indication of decreased mitochondrial respiration and oxidative metabolism, which results in reduced synthesis of ATP production in O. *mossambicus*.

The activity of NADH dehydrogenase was found to be declined in *O. mossambicus* in the present study. NADH dehydrogenase or complex I is the electron donor for the electron transport system, embedded in the inner mitochondrial membrane. NADH dehydrogenase reversibly oxidizes NADH to NAD^+ and transfer electrons to ubiquinone through a redox reaction. This creates an electrochemical gradient to the intermembrane space of mitochondria which is used for ATP production. It is evident from the studies (Sherer *et al*., 2007; Gassner *et al*., 1997) that pesticides (pyridaben, rotenone and fenpyroximate) and insecticides (pyrethroids, permethrin) acts as a potent inhibitor of NADH dehydrogenase activity. Similarly, chemicals like thiazoles, flunarizine and cinnarizine (Veitch and Hue, 1994; Andreani *et al*., 1995) are also inhibitors of mitochondrial NADH dehydrogenase. Such inhibitors are implicated in Parkinson's disease (Sherer *et al.*, 2007). Decreased activity on exposure to BPS indicated the mitochondrial electron transport chain's disturbed function leading to reduced energy production.

Thus it is evident from the current research that BPS disrupted intermediate and mitochondrial metabolism in *O. mossambicus*. Bisphenol S altered mitochondrial metabolism in the hepatic tissue of fish by decreasing the activities of SDH and MDH enzymes and also the respiratory marker enzymes like NADH dehydrogenase and CCO activities and thereby affects TCA cycle. Similarly, significant diversification occurred in the activities of intermediary enzymes by decreasing $G6PO_4$ase, LDH and ICDH and increasing ME, which may have a profound impact on the biochemical machinery of fish.

5. Conclusion

In the present work, all the key enzymes of the TCA cycle were affected when exposed to BPS. Bisphenol S also acts as an uncoupler of oxidative phosphorylation, resulting in shifting aerobic pathway of fish respiration towards anaerobic pathway and inhibiting energy production by suppressing ATP synthesis. This study also showed that fish are highly sensitive to contamination of water, and any disturbances in the activity of metabolic enzyme may lead to drastic changes in their physiological and biochemical processes. In conclusion, the present work indicates that BPS can cause considerable intermediary and mitochondrial metabolism changes in *O. mossambicus*. The causes of these alterations appear to be the result of high energy demands.

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