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BIOCHEMICAL ANALYSIS IN *Tilapia* FINGERLINGS EXPOSED TO BISPHENOL A COMPOUND

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Abstract: **Physiological functions of animals get disturbed on exposure to pollution stress. The better understanding of this mechanism can help us to predict the harmful effects of various chemicals on environment. This study estimates the chronic effect of this compound on biochemical compositions. The sublethal concentration of Bisphenol A treated with *Oreochromis mossambicus* at different time interval and observed that in the liver, kidney, brain and muscle showed declined trend. The decrease in biochemical contents in toxicated fish in the present study also indicates the physiological adaptability of the fish to compensate for compound.**

Keywords: **Fingerlings, biochemical, Bisphenol A and toxicity.**

1. INTRODUCTION

Freshwater biological system comprises of an expansive number of fauna and vegetation in them. These oceanic creatures are exceptionally delicate to even a slight adjustment in nature. They demonstrate an awesome level of wonderful changes when the amphibian biological system is polluted. As fishes are the real tenants of oceanic life, perils to the fish population are a great concern for fishery industry. The reasons for contamination are generally man-made and in this manner the counteractive action and control estimates must be taken however much as could reasonably be expected. Condition being an incorporated framework, any adjustment in its parts would surely disturb its homeostasis. Oceanic biological community is in effect constantly abused unpredictably as the most advantageous waste disposal system. Around 70,000 anthropogenic synthetic compounds are discharged into the sea-going biological communities (Routledge *et al*., 1998, Metzler and Erica, 2001).

Bisphenol A (2,2-bis (4-hydroxyphenyl) propane) (BPA) is generally utilized in the creation of polycarbonate plastics, epoxy saps, warm paper, paints, water-channels, electronic gear, toys, bundling, bottles, medicinal gadgets, surface coatings, printing inks, fire retardants, workstations, cell phones, electronic gadgets, dental sealants, laboratory and hospital equipment, among others (Bjornsdotter *et al*., 2017; Flint *et al*., 2012; Hoekstra *et al*., 2013; Huang *et al*., 2012 and Spagnuolo *et al*., 2017). A portion of these applications were the reasons for human presentation to BPA through nourishment and beverages (Beltifa *et al*., 2017; Ndaw *et al*., 2016) or by inward breath and dermal contact (Hines *et al*., 2017; Li *et al*., 2017; Liu *et al*., 2017). Alteration in the chemical composition of a natural aquatic environment by industrial effluents, more often than not initiate changes in the behavioural, biochemical and pathological aspects of the inhabitants, particularly fish (Edwards, 1973). Normally carbohydrates, proteins and lipids which establish the real parts of the body assume a vital job in development and vitality digestion. Consequently an endeavor has been made in the present examination to know the impacts of a natural compound Bisphenol A in the freshwater fish, *Oreochromis mossambicus* as the target animal to test the harmful impacts with biochemical studies.

2. MATERIALS AND METHODS

FISH STOCK SELECTION, FEED PREPARATION AND FEEDING

Tilapia fingerlings of the same size of a given season, and relatively of uniform size in the range of 10 to 12 cm and weighing 6-8 gm were procured from kurattur lake, chennai. The fish, after conditioning, were oxygen packed in tins and brought to the lab. They were slowly released into tanks, half filled with bore water and seasoned over night. These were maintained in stocking tanks, where the fish were quarantined and acclimatized for four day**.** The fish feed was prepared with rice bran, groundnut oil cake, tapioca powder and mineral mixture (Radhaiah, 1982). Groundnut oil cake was soaked in enough distilled water and minced thoroughly before mixing with rice bran, tapioca powder and mineral mixture. After thorough mixing, the contents were steam cooked in autoclave at 15 psi for 15 minutes, so as to sterilize the mixture. Pellets were prepared by using a hand mincer and shade dried at room temperature (31^0C-33^0C) for 24 hr and later, in a hot air oven at 60^0 to 80^0C for 48 hr. After enough drying, the pellets were stored in air tight containers. The fish were fed daily with pelleted feed at 5% body weight in two split doses, in the morning and evening. Feeding was started one day after the fish were stocked and stopped 24 hr prior to experiment.

MAINTENANCE OF FISH

All the fish were maintained in the glass aquaria of the size 1'L x 2'B x 1'H throughout the period of experimentation. The tanks were disinfected with potassium permanganate thoroughly, before and after use. These tanks were filled with known volume of water per fish (30 litres for 10 fish) and covered with nylon mesh to prevent the mosquitoes breeding in the tanks and also to restrain the fish jumping out. During the period of experimentation, the room temperature fluctuated from 30-32^0C. The water used for the experiment had dissolved oxygen content of 4.4 – 4.8 ml/l and salinity of 0.82 – 0.84ppm. The pH of water was in the range of 7.2 to 7.4.

EXPERIMENTAL FISH

Healthy fish without any observable pathological symptoms were chosen for the experiments. Fish were divided into two groups of ten, where one group as control and another group exposed with Bisphenol A with the concentration of 1mg/L. The Bisphenol A treatment level was based on the 96hr LC_{50} of the former compound in *O. mossambicus,* which was previously determined to be 10mg/L by Chitra and Sajitha (2014). The following parameters have been studied in the untreated control fish, as well as the different experimental groups of fish treated with the toxicants. For biochemical studies, four different tissues muscle, liver, brain and kidney were dissected out carefully and weighed using K-Roy Single pan electrical balance. The dissected tissues were kept in an ice box till taken out for homogenization. Biochemical studies were observed in 7th, 14th, 21st and 28th days.

BIOCHEMICAL STUDIES

TOTAL CARBOHYDRATES

The tissues (muscle, liver, brain, and kidney) were weighed and ground with 3ml of 5% Tri Chloro Acetic acid (TCA). The homogenates were carefully collected and centrifuged for 15 min at 3000 rpm. The clear supernatants were saved for the analysis of total carbohydrates by the method of Carroll *et al*., (1956). 10ml of anthrone reagent was added to 1ml of supernatant and kept in a boiling water bath for 20 min. and later cooled to room temperature. The intensity of the colour developed was read at 620 nm in a photo colorimeter. The values are calibrated from the standard graph, and expressed as milligram per gram wet weight of the tissues.

STATISTICAL ANALYSIS

For all the parameters analysis, five estimations were made and subjected to standard statistical methods to arrive at Mean, Standard Deviation and Test of Significance. The data were statistically evaluated by ANOVA using SPSS program. The results obtained for each experimental group were compared with that of the respective control group utilizing Student's parametric t-test depending on the distribution of data. The results were tabulated and the data represented in suitable tables and histograms. Consolidated tables and graphs based on mean of 5 values were given for all the parameters under the different experimental groups, in an effort to restrain the number of pages. Values of $P<0.05$ were considered significant.

3. RESULTS

The Tilapia fingerlings were exposed to single concentration of Bisphenol A for 28 days. Observations were made from 7-28 days to study the structural and behavioral conditions. Analyses of different parameters were carried out in the fish exposed to SLC of toxicant on 7th, 14th, 21st and 28th day of experimentation. Biochemical studies were carried out from 7-28 days of treatment in the different experimental groups concerned. The results of observations and analyses are given below in detail.

TOTAL CARBOHYDRATE IN LIVER, KIDNEY, BRAIN AND MUSCLE

- The mean value of the carbohydrate content of the normal fish when compared with the Bisphenol A exposed fish was significantly elevated on 7th and 14th day. The elevated level of the sugars was significant with $p<0.05$. The lower value of the average was recorded on 21st and 28th day, which were also significant (Table 1; Fig.1).

- After the treatment with Bisphenol A, sugar content in the kidney was significantly affected. On 7th day, the decline in the carbohydrate content was noticed. When the exposure continued in the same way reduced level in the sugar content was recorded on 14th day, 21st and 28th day (Table 1; Fig.1). The reduction was significant at 5% level when analyzed with Student'tʼ test.

- Treatment of Bisphenol A brought about a significant depletion in the total sugar content of exposed group of fishes with reference to brain tissue. The reduced levels of sugar content were highly significant statistically, when compared to control group of fishes (Table 1; Fig.1).

- The sugar content of the muscle tissue was recorded at 7th, 14th, 21stand 28th days. When fishes treated with Bisphenol A, the sugar content of the muscle tissue was reduced on 7th day, 14th day, 21st day and 28th day. The mean value of the carbohydrate content of the exposed fish was lower when compared to control group. These results were highly significant with $p<0.05$ (Table 1; Fig.1).

TOTAL LIPIDS IN LIVER, KIDNEY, BRAIN AND MUSCLE

- The liver tissue of the normal fish recorded a total lipid content of 22.4 µg/L. On the 7th day of exposure there was a significant elevation in the lipid content of liver. Similarly an increased trend was observed on 14th day. However on the 21st and 28th day of the lipid content showed a decreasing trend in the fish exposed to BPA. These results were significant when analyzed with Student't' test (Table 2; Fig.2).

- The total lipid of the kidney was 17µg/L. in the fish untreated with any toxicant. In the fish treated with BPA, elevated level in the lipid content was noticed on 7th day and 14th day of exposure, while reduced levels were observed on all the other exposure periods (Table 2; Fig.2). These results were significant when analyzed with Student't' test.

- The total lipid was estimated 52.2µg/L. in the brain tissue of the normal fish. Compared to this the lipid content the raised level of the lipid content was recorded on all the other exposure periods i.e. on 7th, 14th, 21st and 28th day, which was significant with $p<0.05$ (Table 2; Fig.2).

- Total lipid content in the muscle of the normal fish was estimated at 90.8 µg/L. Compared to this lipid content eexposure to the BPA had effected a very significant reduction in all the experimental groups of 7th, 14th, 21st and 28th day analyses (Table 2; Fig.2)

TOTAL PROTEINS IN LIVER, KIDNEY, BRAIN AND MUSCLE

- The control fish had 1.74 µg/L protein in the liver tissue compared to this polyphenyl compound Bisphenol A treated fish showed a significant increase in the protein content on all the exposure periods *i.e.* on 7th, 14th, 21st and 28th day (Table 3; Fig.3). The alterations in the protein content were significant, when analyzed statistically.

- The zero day control fish recorded 6.4 µg/L of muscle tissue. After the exposure of Bisphenol A, protein content in the muscle of tilapia fingerlings was significantly depleted on 7th , 14th, 21st day and 28th day. From 7th day onwards the protein content was reduced up to 28th day (Table 3; Fig.3). These variations were significant at $p<0.05$.

- A total of 0.46µg/L of protein content was estimated in the kidney of the normal unexposed fish. An increased trend was observed throughout the period of experimentation from 7th-28th day in the fish exposed with BPA. The alterations in the protein content were significant, when analyzed statistically (Table 3; Fig.3)

- The total protein content of the brain tissue was 0.88 µg/L in the untreated fish compared to the experimental groups which had significant elevation. In all the experimental periods of 7th, 14th, 21st, 28th days the protein content was significantly increased (Table 3; Fig.3) when analyzed with Student't' test.

4. DISCUSSION

Bisphenol A (BPA) is a vital compound mostly utilized underway of epoxy tars and polycarbonates, and is particularly inexhaustible in PVC plastics (Vandenberg *et al*., 2009). It is likewise present in paper coatings, dental sealants, glues, reusable containers (e.g., infant bottles), nourishment and refreshment bundling, fire retardants, added substance in different plastics and building materials (Staples *et al*., 1998). BPA is one of the most noteworthy volume synthetic concoctions created around the world. Worldwide BPA creation limit in 2003 was 2.2 million metric tons (more than 6.4 billion pounds), with a 6 - 10% development sought after expected every year (Burridge, 2003). With the expanding interest for polycarbonates and epoxy tars the market for BPA has been developing and worldwide interest has developed from 3.9 million tons in 2006 to around 5 million tons in 2010 (Tsai, 2006).

The study of the biochemical constituents of tissues of an organism is an important feature that provides key for diagnosis of the physiological state of fish. Alteration in physiological, biochemical and enzymological parameters of toxicant treated fish has recently emerged as important tools for water quality assessment in the field of environmental toxicology. In the present investigation, total sugar content in the muscle, liver, brain and kidney revealed a mixed trend in the different experimental groups and different days of the exposure. There was a lifted level of starches on seventh and fourteenth day of introduction in liver tissue; whereas decrease in 21st and 28th day presentation with BPA when compared to control groups.This shows the severe toxic nature of BPA during the short term exposure period itself.

In the present examination, add up to sugar content in the muscle, liver, brain and kidney uncovered a blended pattern in the diverse exploratory gatherings at various long periods of exposure. Similar perceptions of blended pattern in the sugar content were accounted for in the muscle, liver, cerebrum and kidney of *Catla catla* treated with overwhelming metal and fungicide (Sujatha, 2006). Depletion in total protein could be due to augmented proteolysis and possible utilization of their product for metabolic purpose, decline in protein content may be related to impaired food intake, increased energy cost of homeostasis, tissue repair and detoxification mechanism during stress (Saraswathi *et al*., 2004).

The decrease in the sugar substance of muscle, cerebrum and kidney tissues could consequently be credited to the expanded rate of glycolysis in tissues under stress. Kumari and Ram Kumar (1997) and Sastry and Siddiqui (1983) likewise detailed a huge abatement in the sugar substance of muscle, liver, mind and heart tissues of *Channa punctatus* inhabiting the dirtied waters of Hussain Sagar pool of Hyderabad. Acute toxicity studies of BPA on the grass carp fry showed significant changes in the biochemical constituents of Grass Carp, *Ctenopharyngodon idella*. This finding is supported by data of acute toxicity on other fish Chen *et al*., (2012). Fishes use glycogen, the storage form of carbohydrates for their immediate energy requirement during stress (Vijayan and Moon, 1992). Liver and muscle are the two active sites where storage and metabolism of carbohydrate reserves take place. Generally, depletion in carbohydrate content is directly proportional to the exposure period of the toxicant. Carbohydrates are the chief sources of energy for any organism (Saravanan *et al.*, 2000). These are found in large amounts in the liver and muscle tissues and in small amounts in kidney and brain. A diminishing in the glucose substance of the liver, muscle and kidney tissue of *Clarias batrachus* was seen on presentation to sodium arsenite (Nimaichandra *et al*., 2005). Neethirajan and Madhavan (2004) likewise detailed changes in the sugar of liver, muscle, and gill tissue of *Mystus vittatus* when presented to sumidon.

Protein showed a trend of increasing and decreasing activity in the different experimental groups. As far as lipids were concerned there was a reduction in muscle tissues of 7th, 14th, 21st and 28th day experimental group. Increase in the protein content was in accordance with the reduction of sugar content and lipid content suggesting gluconeogenic pathway utilizing lipid and proteins to compensate for the loss of sugars.The rise and depletion in the levels are highly significant. The trend shows very high metabolic rate that may be due to the initial stress exerted on the fish which were exposed to toxicant. Similar observations of decrease in the protein content were reported in the muscle of *Channa punctatus* treated with heavy metal (Jana and Bandyopadhyay, 1987), in the muscle of Puntius stigma after exposure with pesticides (Killare and Wagh, 1989), in the muscle, liver and intestine of *Cyprinus carpio* treated with textile mill effluent (Rajan, 1990), in the muscle of *Oreochromis mossambicus* due to the effect of phenol (Ravichandran *et al*., 1994), in *Etroplus maculates* exposed to Ekalux (Nelson and Sunil Kumar, 1996) and in *Anabas testudineus* under the influence of alloxan monohydrate (Bhaskar, 1997) .

Proteins are the most critical vitality source to save amid ceaseless time of pressure (Yadav *et al*., 2003). Creature presented to toxicants even at the sub-deadly levels encounter extraordinary worry at the metabolic level amid the time of detoxification of the toxicant (Yadav *et al*., 2003). Proteins are associated with major physiological occasions and hence, the evaluation of the protein substance can be considered as a symptomatic instrument to decide the physiological periods of living being. Exhaustion of protein content has been seen in the muscle, digestive system and cerebrum of the fish, catla, because of poisonous quality (Ilavazhahan *et al*., 2012). Significant decrease was observed with protein content in muscle, liver, brain and kidney of *Cirrhinus mrigala* exposed with sub-lethal concentrations of cypermethrin (Neelima *et al*., 2017).

Lipid profiles are imperative pointers of wellbeing and ordinary metabolic state in any creature. Lipid stores act the hero of creatures on occasion of emergency like pressure and starvation. They are engaged with the transformation to sugars or proteins relying upon the need to balance out the metabolic procedures and for physiological procedure to continue forever without trade off. Raised lipid substances are oftentimes connected with expanded bio concentration, of lipophilic toxicants, which is typically associated with upgraded poisonous quality of these mixes. High lipid stores may apply defensive impacts by expelling and inactivating natural synthetic compounds from the digestion or even effectively sequestering them, along these lines enhancing toxicant resilience and resistance. The irregular gathering of fats in exploratory creatures could be because of initiated unevenness between fat generation and utilization. It could be hypothesized that greasy liver changes are credited generally to the lessened creation of lipoproteins. The values of lipid increased as the dose and exposure time increased but this increase is significant at P=0.05. This increase may be due to the fact that BPA mimic estrogen like activity and estrogen is a known obesogen (Schneider *et al*., 1979). Phrakonkham *et al*., (2008) reported that dietary xenoestrogens increased the expression of adipocyte differentiation genes. It was observed that BPA also enhanced glucose transport in adipocytes (Sakurai *et al*., 2004), which in turn may contribute to lipogenesis. It has been proposed that intense or interminable treatment of pesticide causes biochemical modification in the organs associated with detoxification systems (Shobha Rani *et al*., 2001 and Prabhakar *et al*., 2002). Substantial metal-instigated biochemical change in rohuwas announced by Neha *et al*. (2004) and disabled sugar and protein digestion (Sivakumar *et al*., 1997). De Smet and Blust (2001) have revealed that proteolysis is proposed to expand the job of proteins in the vitality creation amid cadmium stress. A decrease of starch, proteins and lipids were seen in freshwater fish Labeo rohita affected by substantial metal, lead (Neha *et al*., 2004). The profluent from color (Baskaran *et al*., 1989), tannery (Ambrose *et al*., 1994) and refinery (Maruthi and Rao, 2000) detailed a serious decrease in the sugar, protein and lipid substance among the fish population. Lemos *et al*., (2010) found BPA induce differential protein expression in a terrestrial isopod. Present study showed that glycogen level increased significantly with exposure of BPA while cholesterol level increase was not significant. Published experimental work on acute toxicity of BPA and its effects on fish biochemistry are very limited. Further work with BPA acute toxicity testing in fish and its effects on biochemistry will be very useful in accessing possible ecological risk of BPA.

5. CONCLUSION

The biochemical parameter like total sugars, proteins, lipids in the different tissue provides crucial information about the quality of the environment. The significant changes in the tissue bring about the alterations in the histological organization of vital organs of fishes due to the exposure of BPA. The result of one or more toxic chemicals reacting sufficiently in high concentrations in body cells will alter the biochemical components of the aquatic organisms. The behavioral pathology is an indicator of the harmful effect of pollutants. The study of the biochemical constituents of tissues of an organism is an important feature that provides key for diagnosis of the physiological state of fish. Alteration in biochemical parameters of toxicant treated fish has recently emerged as important tools for water quality assessment in the field of environmental toxicology was concluded.

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APPENDICES-A

List of Table:

Table 1: Changes in the Carbohydrate, content in liver, kidney, brain and muscles of *Oreochromis mossambicus* exposed to lower sub lethal concentration (µg/L) of Bisphenol A for 7, 14, 21 and 28 day

		7 day	14 day	21 day	28 day
Liver	control	0.115 ± 0.002	0.122 ± 0.003	0.125 ± 0.002	0.130 ± 0.006
	treated	0.151 ± 0.003	0.148 ± 0.011	0.016 ± 0.007	0.79 ± 0.002
Kidney	control	0.102 ± 0.007	0.102 ± 0.003	0.105 ± 0.003	0.104 ± 0.004
	treated	0.076 ± 0.004	0.08 ± 0.004	0.099 ± 0.004	0.092 ± 0.003
Brain	control	0.1 ± 0.005	0.11 ± 0.005	0.114 ± 0.007	0.117 ± 0.008
	treated	0.088 ± 0.002	0.102 ± 0.001	0.104 0.002	0.104 ± 0.004
Muscle	control	0.1 ±0.003	0.101 ± 0.006	0.1 ± 0.008	0.111 ± 0.005
	treated	0.097 ± 0.004	0.091 ± 0.003	0.088 ± 0.003	0.068 ± 0.002
Results are mean (X ± SD) of 5 observations indicates the standard deviation values and are significant at P < 0.05					

Table 2: Changes in the Lipid content in liver, kidney, brain and muscles of *Oreochromis mossambicus* exposed to lower sub lethal concentration (µg/L) of Bisphenol A for 7, 14, 21 and 28 day

		7 day	14 day	21 day	28 day
Liver	control	22.4 ±0.12	55.2 ± 0.16	139.2 ± 0.19	172.6 ± 0.13
	treated	33.4 ± 0.10	61.2 ± 0.28	68 ± 0.16	76.2 ± 0.14
Kidney	control	17 ±0.02	44.8 ± 0.16	86.4 ± 0.23	107.8 ± 0.23
	treated	60.8 ± 0.14	62.8 ± 0.25	74.8 ± 0.13	99.8 ± 0.31
Brain	control	52.2 ± 0.21	80.4 ± 0.23	97.4 ± 0.24	93.2 ± 0.12
	treated	122.8 ± 0.15	115.4 ± 0.11	114.2 ± 0.18	117.2 ± 0.24
Muscle	control	90.8 ± 0.19	122.2 ± 0.18	290.4 ± 0.21	504 ± 0.42
	treated	61.2 ± 0.22	83.6 ± 0.17	69.8 ± 0.17	73.8 ± 0.17
Results are mean (X ± SD) of 5 observations indicates the standard deviation values and are significant at P < 0.05					

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Table 3: Changes in the Protein content in liver, kidney, brain and muscles of *Oreochromis mossambicus* exposed to lower sub lethal concentration (µg/L) of Bisphenol A for 7, 14, 21 and 28 days

		7 day	14 day	21 day	28 day
Liver	control	1.74 ± 0.008	1.9 ± 0.003	1.95 ± 0.004	1.96 ± 0.006
	treated	3.96 ± 0.02	5.56 ± 0.018	5.44 ± 0.015	5.94 ± 0.015
Kidney	control	0.46 ± 0.003	0.51 ± 0.003	0.51 ± 0.003	0.45 ± 0.021
	treated	1.48 ± 0.007	0.92 ± 0.004	1.05 ± 0.006	0.62 ± 0.016
Brain	control	0.88 ± 0.006	0.81 ± 0.005	0.82 ± 0.004	0.68 ± 0.012
	treated	3.72 ± 0.014	2.5 ± 0.012	1.69 ± 0.007	0.97 ± 0.015
Muscle	control	6.4 ± 0.021	5.62 ± 0. 023	4.5 ± 0.003	3.54 ± 0.021
	treated	1.44 ± 0.006	1.98 ± 0.007	2.54 ± 0.002	3.18 ± 0.014
Results are mean (X ± SD) of 5 observations indicates the standard deviation values and are significant at P < 0.05					

List of Figure:

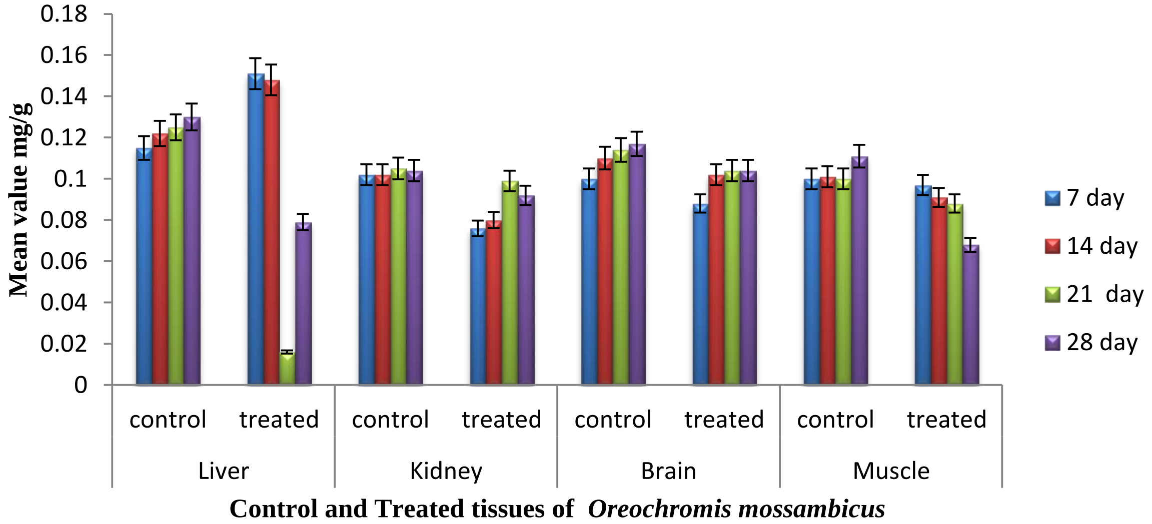

Fig 1: Carbohydrate content in liver, kidney, brain and muscles of *Oreochromis mossambicus* exposed to lower sub lethal concentration (µg/L) of Bisphenol A for 7, 14, 21 and 28 day

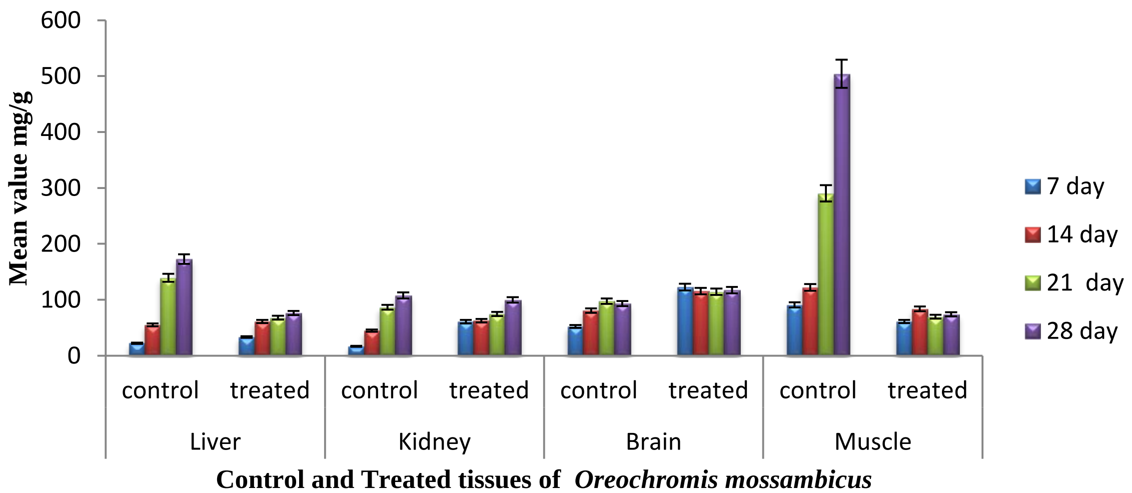

Fig 2: Lipid content in liver, kidney, brain and muscles of *Oreochromis mossambicus* exposed to lower sub lethal concentration (µg/L) of Bisphenol A for 7, 14, 21 and 28 days

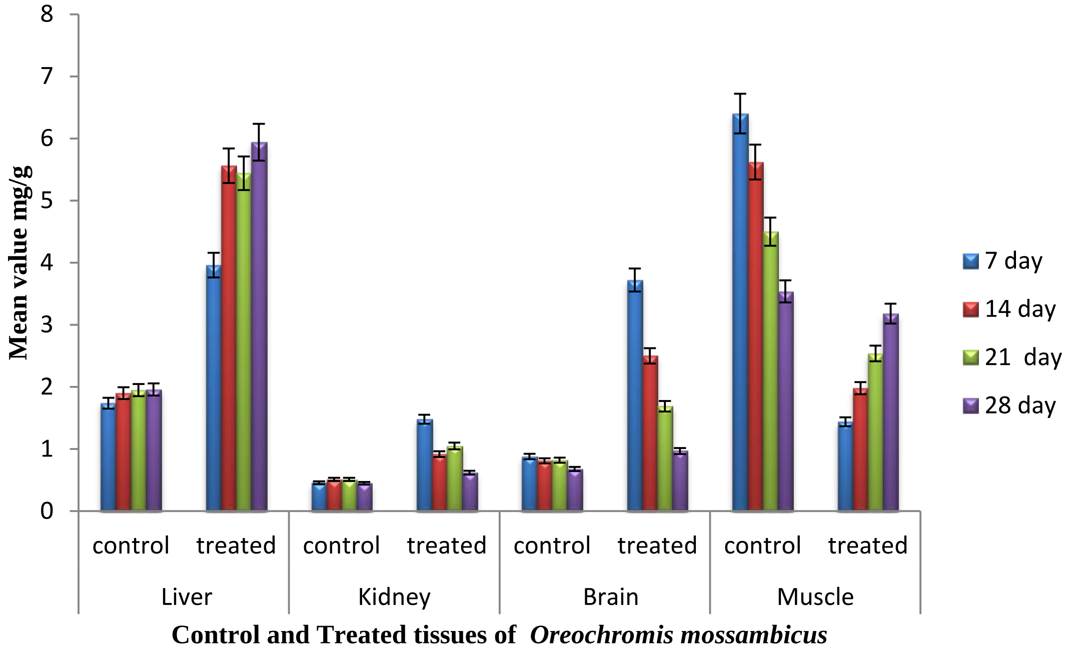

Fig 3: Protein content in liver, kidney, brain and muscles of *Oreochromis mossambicus* exposed to lower sub lethal concentration (µg/L) of Bisphenol A for 7, 14, 21 and 28 days