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AROCLOR-1254 INDUCED OXIDATIVE STRESS RESPONES IN A FRESHWATER FISH *ANABAS TESTUDINEUS* (BLOCH)

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Abstract: Aroclor-1254, one of the important polychlorinated biphenyls (PCBs), widely used in the production of plastics, adhesives, paints, varnishes, newsprint, pesticide extenders, lubricating oils and coolants in transformers. Lower levels of PCBs are broadly prevalent in foodstuffs, and a great deal of attention has been paid to these PCBs, especially higher chlorinated ones, those more resistant to metabolic transformation, and those from certain food sources, especially in fish. This compound has the potential of causing neurological problem, impairment in the reproductive system, hepatic cancer, alterations in the enzyme machinery, developmental abnormalities and imbalance in the endocrine system. Hence an attempt was made to examine the toxicity of aroclor 1254 on the antioxidant system of *Anabas testudineus*. Fish ware administered with three doses (15, 30 and 45µg/g bodyweight) of aroclor 1245 for 10 20 and 30 days. All the three doses of aroclor 1254 significantly reduced the activity of SOD and increased the activity of CAT, GST and GPX after 10, 20 and 30 days. Significant increment in the content of MDA and CD could be observed due to aroclor 1254 administration. Aroclor-1254 in liver exhibited serious alterations like vacuolation, melanomacrophagecentre etc. Fragmentation in DNA could be observed in all doses studied. Therefore, the current results are relevant in understanding the toxicity of aroclor-1254 at the level of DNA and contribute knowledge about the possible hazardous effects in humans by consuming the contaminated fish.

Key words: Polychlorinated biphenyles, superoxide dismutase, lipid peroxidation, malondialdehyde, conjugatediene, melanomacrophagecentre, DNA damage.

INTRODUCTION

Aquatic contamination has increased worldwide as a result of domestic and industrial wastes. Enormous quantities of wastes with oxidative potential are daily disposed into the water bodies. Different components in the wastes are reacted together and with the aquatic organisms lead to biochemical and physiological problems in them.Environmental impacts caused by trace organic pollutants such as poly chlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) have receivedincreasing attention due to their persistency and tendency to bioaccumulatethrough the food chains (Lee *et al.* 2001). Polychlorinated biphenyls (PCBs) are widely spread environmental organic pollutants, which have been identified as contaminants in almost every component of the ecosystem including fish, wildlife and human adipose tissue, breast milk and serum (Anne *et al*., 2007). A primary biochemical effect of PCBs is the induction of hepatic mixed function oxidase systems(Litterest *et al*., 1972; Lindman *et al*., 1976; Elcombe *et al*., 1979; Voss *et al*., 1982), with resultant alterations in xenobiotic metabolism.

Aroclor-1254 is one of the important PCBs widely used in the production of plastics, adhesives, wood and floor finishers, paints and varnishes

etc. It can cause oxidative damage to biomolecules in the form of lipid peroxidation, modulation of antioxidant enzymes and oxidative stress (Banudevi *et al*., 2006). The imbalance between the prooxidants and antioxidants leads to the accumulation of prooxidants and the animal feels oxidative stress. Aroclor-1254 in high concentration delayed the plasma cortisol response to stress. This seriously affected the winter sleep of Arctic charr (Jorgensen *et al*., 2002). Aroclor 1254 exposure resulted in imbalance in the function of thyroid hormone in Atlantic croaker (Kimberly *et al*., 2006). According to Harry *et al* (1982) aroclor-1254 has detrimental effect on the physiology of Atlantic cod, *Gadus morhua*. Lower chlorinated PCBs produce reactive oxygen species (ROS) and intracellular oxidative stress (Oakley *et al*., 1996). Free radicals, particularly hydroxyl radicals, may produce 8-oxodeoxyguanosine (8-oxodG), a DNA lesion that is highly mutagenic, producing G->T transversions (Marnett and Burcham, 1993). Hydroxyl radicals can also attack fatty acids (linoleic acid, linolinic acid, oleic acid, etc) and form lipid peroxidation-derived enals, such as acrolein, crotonaldehyde, *trans*-4-OH-2-nonenal (4-HNE), and malondialdehyde (MDA) (Nair *et al*., 1999). These products can then modify DNA bases, resulting in cyclic adduct by interaction of their difunctional groups with NH2 group in dA, dG or dC residues in DNA (Chaudhary*et al*., 1994; Chung *et al*., 1996; Winter *et al*., 1986). These cyclic adducts are mutagenic, producing base substitutions and deletions, for example G->T mutations from propano-dG and C->A mutations from various etheno adducts (Basu *et al*., 1993; Marnett and Burcham, 1993; Nath *et al*., 1996). Damage of hepatic tissue is one of the consequences of oxidative stress. According to Parvathy *et al*. (2011) chromium has the potential to cause atrophy in liver tissue. Oxidative stress is one of the reasons of DNA damage in fish.DNA adducts are formed by bulky genotoxins, such as aflatoxinB1 (AFB) where Busby *et al*. (1984) reported that in the liver microsomes, AFB is oxidised to its reactive epoxide forming exo AFB-8, 9 epoxide. This subsequently links itself to DNA and exhibits the mutagenicity (Laskyand Magder, 1997). In the light of the above described facts an attempt was made to examine the impact of a polychlorinated byphenyl(PCB), aroclor 1254 on the antioxidant system in a freshwater fish *Anabas testudineus*.

MATERIALS AND METHODS

Experimental Design

Adult fish (*A.testudineus*) were purchased from local suppliers, brought to the laboratory and kept in large storage tanks. They were acclimated for 3 weeks and fed on alternate day with commercially available fish feed.Acclimatized fish weighing 35±5g were divided into ten groups consisting eight in each group were transferred to separate aquarium tanks. The LC_{50} of aroclor was determined as 120µg/g bodyweight. So sub lethal doses were selected as 15, 30 and 45µg/g bodyweight of aroclor-1254 in corn oil as vehicle. The first three groups of fish were injected intraperitoneally (ip) 15µg/g bodyweight aroclor 1254 for 10, 20 and 30 days. The other three groups were administred (ip) 30µg/g bodyweight aroclor 1254 for 10, 20 and 30 days. The next three groups were injected (ip) with 45µg/g bodyweight aroclor 1254 for 10, 20 and 30 days and maintained as experimental group. Tenth group of fish were injected with corn oil only and considered as control. After stipulated periods of injection, fish were decapitated and liver was excised, a part of the tissue was fixed in aqueous Bouin's fluid for light microscopical study and remaining liver was kept at -80^0 C for enzyme assay and DNA fragmentation studies.

Biochemical Analysis

Chemicals were of analytical grade and purchased from Sigma chemicals U.S.A. Protein was estimated according to the protocol of Bradford (1976). For assaying all antioxidant enzymes, 100 mg tissue was homogenized in 2 ml sucrose solution (0.025M) and centrifuged at 10,000g for 10 min and the supernatant was taken as extract.

Assay of Superoxide dismutase (SOD) (EC.1.15.1.1)

SOD activity was estimated according to the protocol of Kakkar *et al* (1984).The assay mixture contained 1.2 ml of 0.052 M sodium pyrophosphate buffer (pH 8.3), 0.1 ml of 186mM phenazinemethosulphate , 0.3 ml of 300mM nitro blue tetrazolium , 0.2 ml NADH (780 mM), appropriately diluted enzyme preparation and distilled water in a volume of 3 ml. Addition of NADH started the reaction. After incubation at 30^{0}C for 90 seconds, the reaction was stopped by the addition of 1 ml of glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4 ml n-butanol. The mixture was allowed to stand for 10 min, centrifuged and butanol layer was pipetted out. Colour intensity of the chromogen in butanol was measured at 560 nm against butanol (blank) using UV spectrophotometer. One unit of enzyme activity was defined as the enzyme concentration required to inhibit the optical density at 560 nm of chromogen production by 50% in one minute under the assay conditions. The specific activity was expressed as IU mg protein-1.

Assay of Catalase (CAT) (EC.1.11.1.6)

Catalase activity was measured according to the method of Maehly*et al* (1954) The reaction mixture contained phosphate buffer (0.01 M, pH 7.0), 2 mM H_2O_2 and 0.1 ml of approximately diluted extract, prepared by homogenizing the tissue in 10 mM buffer.A system devoid of extract constituted blank. Decrease in absorbance was measured at 230 nm for 2 min. The specific activity was expressed in terms of n moles of H_2O_2 liberated / min / mg protein.

Assay of Glutathione –S- transferase (GST) (EC.2.5.1.18)

The activity of GST was measured according to the protocol ofHabig *et al.* (1974). The cocktail contained 1 ml phosphate buffer (0.5 M, pH 6.5), 100 µl of 30 mM, 1-chloro, 2, 4, dinitrobenzene (CDNB), 0.1 ml of 30 mM reduced glutathione and 100 ìl extract. The increase in optical density of the enzyme was measured against that of the blank at 340 nm for 2 min. Enzyme activity was expressed as n moles of CDNB-GSH conjugate formed / min / mg protein for tissue samples.

Assay of Glutathione peroxidase (GPX) (EC.2.5.1.18)

GPX was measured according to Lawrence and Burk (1976) with slight modification (Agergaard and Jensen, (1982). The assay volume contained 2 ml of .01 M(pH7.4) with 300µl EDTA, 300µl of 1mM sodium azide, 100µl of 0.1 M NADPH,,100µl of 1mM reduced glutathione and 200µl tissue extract. Blank contained all the reagents except tissue extract. Change in absorbance was measured at 340 nm at 15 seconds interval for 1 minute. Enzyme activity was expressed as IUmg protein^{-1}.

Assay of Malonedialdehyde (MDA)

Malonedialdehyde was measured according to the protocol of Nichans and Samuelson (1968). 100 mg tissue was homogenized in 1 ml trisHCl buffer (pH 7.5) and centrifuged at 10,000g for 10 min. The supernatant was taken as extract. 1ml homogenate was added to 2 ml TBA.TCA.HCl and heated in boiling water bath for 15 min. Then it was centrifuged at 1000g for 10 min and absorbance measured at 535 nm. The blank consisted of 2 ml TBA.TCA.HClreagent.The level of MDA was expressed as µ moles/g tissue.

Assay of Conjugate diene (CD)

It was measured according to the method of Recknagel and Ghoshal(1996). The reaction mixture contained 1ml tissue homogenate, 5ml chloroform/methanol(2:1). It was centrifuged at 1000 rpm for 10 minutes; 3mlof lower layer was carefullupippeted out and dried at 45^{0}C in a water bath. To this, 1.5 ml of cyclohexane was added and absorbance measured at 233nm. concentration of CD was expressed as µmoles/g tissue.

Histology

After fixation in Bouins fluid, the liver was passed through graded alcohol series (30%, 50%, 70%, 90% and 100%) for dehydration.Then it was cleared in benzene for 1 minute. After clearing,

the tissue was subjected to infiltration by embedding in paraffin wax (56^{0}C) for 20 minutes.After embedding sectioning of the block was carried out. Sections were cut at 5µ thickness using a rotary microtome (Leica, Germany). Sections were spread on a clean glass slide coated with Mayer's albumen.

The slides were rendered transparent with xylene and subjected to hydration through graded alcohol series (100%, 90%, 70%, 50%, 30% and distilled water). Then they were stained with haematoxilin and eosin. After staining they were subjected to dehydration (30%, 50%, 70%, 90% and 100%) and then cleared in xylene allowed for air dry. The dried slides were mounted in DPX. The stained sections were observed under a binocular research microscope attached with CCDcamera(Leica, Germany).Appropriate sections and portions of tissues were photographed using Leica image analyzer software.

Agarose gel electrophoretic detection of DNA damage.

Hepatic DNA isolation and electrophoresis was carried out according to the method of Iwasa *et al.* (1996) with some modifications. Briefly, the homogenized liver tissue was lysed with buffer containing 10 mMTris-HCl, 1 mMEDTA, 1% SDS and 100 mg/ml Proteinase K and kept in a water bath for 12 hr at 37^{0}C. DNA was extracted twice with equal volume of phenol :chloroform:isoamyl alcohol (25:24:1). To the aqueous phase, 3 M sodium acetate was added and the DNA was precipitated with chilled isopropanol. Following a 70% ethanol wash, the precipitated DNA was resuspended in Tris- EDTA buffer and was electrophoresed in 1% agarose containing.5µg/ mlethidium bromide. The DNA damage were visualized under gel doc(Gelstan, Medicare,Germany).The migration distance of the DNA moleculefrom the top of the gel was used as a measure of DNA damage.It is recognized that the highly fragmentedlow molecular weight DNA strand will migrate farther than non damagedhigh molecular weight DNA strands.

Statistical analysis

Data collected from each group was analyzed by one way analysis of variance using SPSS package. Groups that were not significantly different in Duncan's (1955) multiple range tests were considered homogenous. Difference was considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Administration of all three doses of aroclor-1254 for 10, 20 and 30 days significantly decreased the activity of SOD (Fig.1) But significant increment could be noticed in the activity of CAT(Fig.2), GST (Fig.3), GPX (Fig. 4)in all the three concentrations of aroclor 1254 after 10, 20 and 30 days. The level of lipid peroxidation products MDA (Fig. 5) and CD (Fig. 6) was found to be increased after 10,20 and 30 days at all the three doses of aroclor 1254. Hepatic tissue of control fish showed parenchymatous cell with intact nucleus, absence of vacuolation and limited melanomacrophagecentre(MMC) (Fig.7). Aroclor-1254 (15 and 30µg/g body weight) administered liver tissue showed loss of normal cell architecture, more melanomacrophagecentre, pyknotic nucleus, disrupted blood vessel and vacant blood vessel(BV) after 30 days (Fig. 8,9 and10). But administration of 45µg/g body weight of aroclor-1254 resulted in the development of intensevacuolation and disrupted hepatic duct (Fig. 11). Total disruption of hepatic tissue was the result of the administration of maximum dose of aroclor 1254 (Fig. 12). Aroclor 1254 induced DNA fragmentation at all concentrations. But the maximum DNA damage could be found only after 30 days of exposure (Figs. 13&14).

Only limited studies were carried out on the effect of aroclor 1254 on fish. In the present study the administration of aroclor 1254significantly decreased the activity of SOD, indicating the lower effectiveness of SOD in removing free radicals. The same results were obtained in the fish, rainbow trout exposed to lead and copper (Burhan *et al*., 2008). Administration of aroclor-1254 resulted significantly enhanced the activity

Fig. Effect of aroclor-1254 on the antioxidant enzymes and the level of lipid peroxidation products.

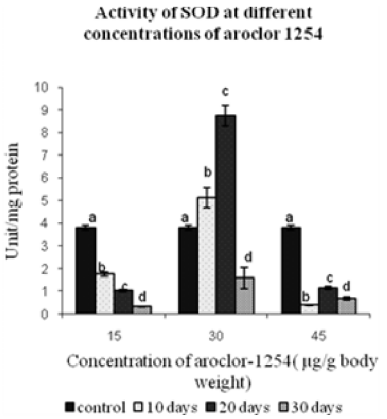

Fig. 1.

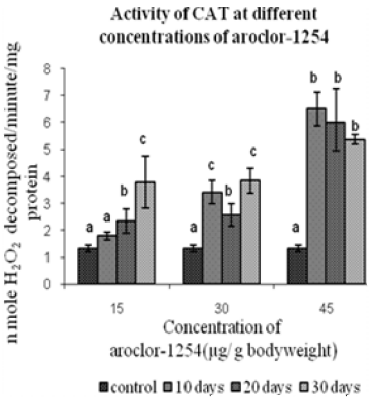

Fig. 2.

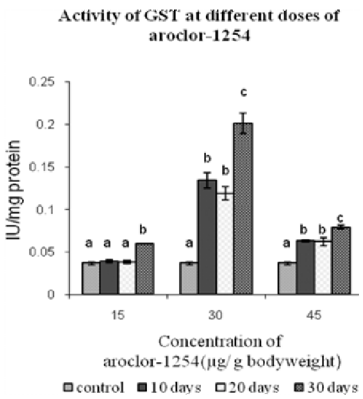

Fig. 3.

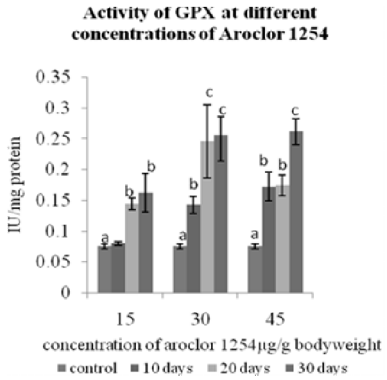

Fig. 4.

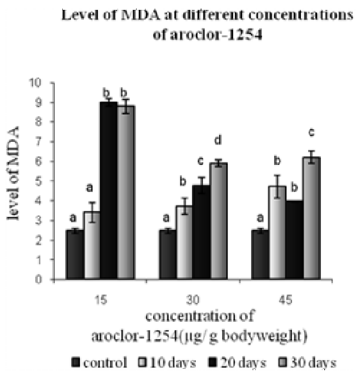

Fig. 5.

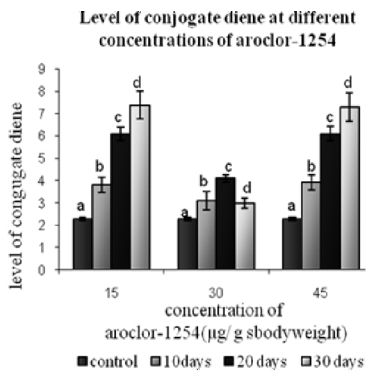

Fig. 6.

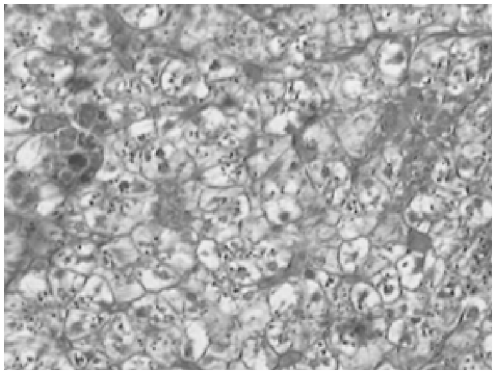

Fig. 7. Control liver with normal cell (x40)

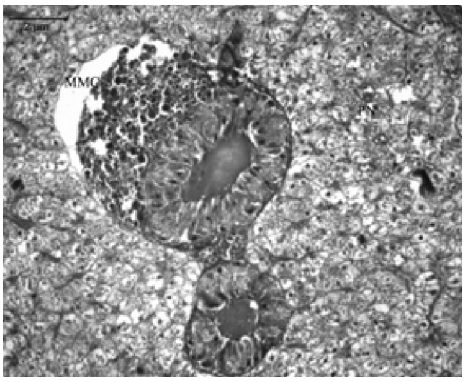

Fig8.15µg/g bw with melanomacrophage centre (MMC)

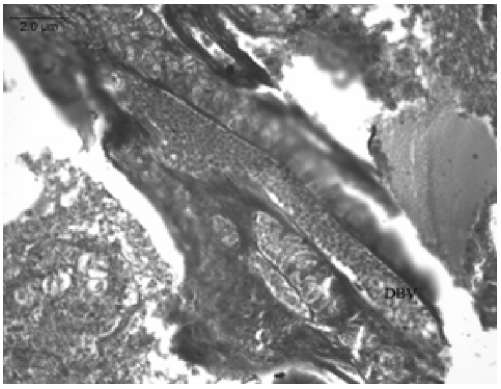

Fig. 9. liver (30µg/g body weight) with disrupted blood vessel (DBV)

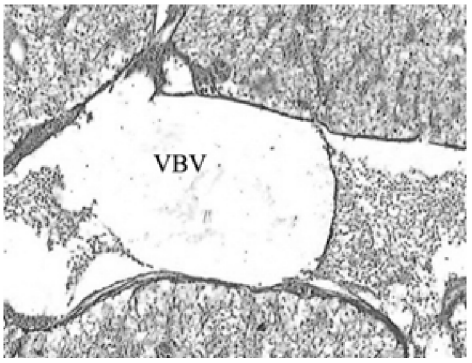

Fig. 10. liver tissue (30µg/g bodyweight) 20 and 30 days showing vacant blood vessel (VBV) (10x)

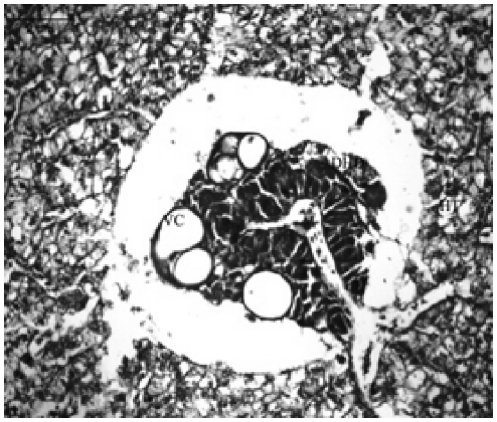

Fig. 11. 45µg/g bodyweight 20 and 30 days showing vacuolation

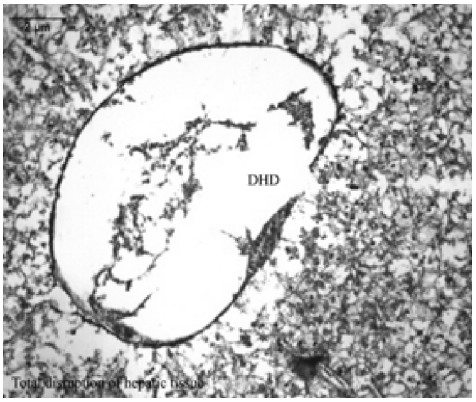

Fig. 12. total disruption of hepatic tissue

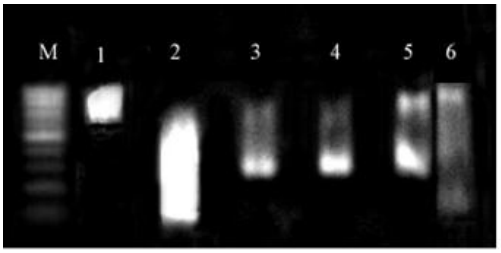

Fig. 13. Effect of aroclor 1254 on DNA

M- Marker, lane 1 - control, lane 2- 15µg 30 days, lane 3- 15µg 20 days, lane 4- 30µg 10 days, lane 5 – 30µg 20 days, lane 6- 30µg 30 days.

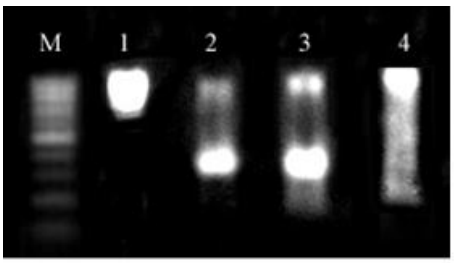

Fig. 14. Effect of 45µg aroclor 1254 on DNA

M- Marker, lane 1 - control, lane 2- 10 days, lane 3- 20 days, lane 4- 30 days

of CAT, GST and GPX. This activation suggests that large amounts of peroxide were generated for overcoming the ability of CAT, GST and GPX to neutralize reactive oxygen species production. The activation of antioxidant systems in response to exposure to pollutants has been reported in various fish tissues (Amado *et al*., 2006; Shaik*et al*). The induction of catalase in the liver was an adaptive response of the cells to mitigate the toxicity of aroclor 1254.The glutathione-S-transferase detoxifies a number of environmental carcinogens, reactive nucleophile, and epoxides intermediates. The increased glutathione-S-transferase assay was suggested as a useful tool for biomonitoring oxidative stress (Di Giulio *et al*., 1993).The liver is a major target organ for ingested oxidants that increases glutathione peroxidase activity. This probably reflects an adaptation to the oxidative conditions to which the fish have been exposed Lenartova *et al*., 1997. Significant increase in glutathione peroxidase activity was observed predominantly in the liver indicates the protective role of the enzyme against lipid peroxidation.

Increases in the level of lipid peroxidation products under the influence of aroclor-1254 indicated that this toxic compound generated reactive oxygen species in the liver and the antioxidant system is defective to remove them. Exposure of cadmium, mercury, aroclor etc. resulted increased malondialdehyde in hepatic and ovarian tissues and caused extensive lipid peroxidation in fishes. (Wofford, 1993, Bakel *et al*., 1997; Romeo *et al*., 2000). Many studies have demonstrated that lipid peroxidation and oxidative stress increase in tissues of different species of aquatic organisms, because of being exposed to environmental stress (Winston *et al*., 1991). The increased level of malondialdehyde observed in the liver of anabas agreed with the previous investigation carried out with tilapia that had been exposed to microcystins (Jose *et al*., 2005) Alterations in the liver tissue clearly indicated the toxic nature of the compound. This was also evident in the study of (Sivarajah *et al*., 2006). Intense vacoulation and disrupted blood vessels were another noticable damage of liver tissue. These pathologies are consistent with those of the study of (Thophon *et al.*,2003). Lawenze *et al.* (2010) reported that an organoclorine, gammalin 20 has the potential to impair the liver tissue by generating pyknotic nucleus and vacoulation. This is in conformity with our result; in which also vacuoles and nuclear pyknosis could be seen. In the present study, the higher levels of DNA damage observed in aroclor 1254 administered fish could be attributed to the ability of aroclor 1254 to induce the generation of free radicals. This is consistent with the study of **(**Madhusudhanan *et al*., 2006),they report a direct correlation between aflatoxin B1-induced oxidant generation and resultant DNA damage in the fish *Labeorohita*. Aroclor-1254 and Dioxin resulted in DNA strand breaks and apoptosis in erythrocytes in the fish,

Anguilla anguilla. These are known to act through oxygen free radical mechanism (Nigro *et al*., 2002). This is in confirmity with our results. Since genetic material is the basic unit of heredity, there is a chance to inherit the abnormalities caused by PCBs in the organism to the next generation. So molecular level studies deserves importance in this regard.

CONCLUSIONS

In the present study it is evident that the influence of aroclor-1254 adversely affected the normal functioning of liver by disrupting the antioxidant mechanism, lead to increased lipid peroxidation and tissue damage and thereby damage to the hereditary material DNA. This may happen in other animals and humans by consuming the contaminated fish. Therefore, the current results are relevant in examining the toxicity of aroclor-1254 in causing damage to the vital macromolecule DNA and may contribute to knowledge about oxidative stress in fish. So the imbalance in the antioxidant system, hepatic damage and subsequent DNA damage can be taken as biomarkers of aquatic pollution. Thus there is an urgent need to monitor aroclor 1254 as well as other persistent organic pollutants in our rapidly changing terrestrial and aquatic environment for the maintenance of economically important fish species.

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