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RESEARCH **P**APER

Profiling of antioxidant enzymes in cat fish (*Clarias batrachus*) exposed to phenolic compounds

AMIT ALEXANDER CHARAN[1], ARADHANA IRENE CHARAN[1], OM PRAKASH VERMA[1] **AND** SYED SUAIB NAUSHAD[2]

[1]Department of Molecular and Cellular Engineering, Sam Higginbottom Institute of Agriculture, Technology and Sciences, ALLAHABAD (U.P.) INDIA
[2]Department of Biochemistry and Biochemical Engineering, Sam Higginbottom Institute of Agriculture, Technology and Sciences, ALLAHABAD (U.P.) INDIA
Email : amit.charan@shiats.edu.in

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Pollution of water sources due to chemicals plays a primary role in the destruction of ecosystems but chemical analyses alone may not suffice to describe the adverse effects of the complex mixtures of chemicals present at contaminated sites. The potential utility of biomarkers for monitoring both environmental quality and the health of organisms inhabiting in the polluted ecosystems has received increasing attention during the last years. In the present investigation, the antioxidant profile of *Clarias batrachus*, a fresh water fish was determined by evaluation of antioxidant enzymes, SOD activity, catalase activity, glutathione peroxidase activity, glutathione-S-transferase activity, reduced glutathione, level of conjugated dienes, hydrogen peroxidase activity and malondialdehyde level. The studies showed that in fishes after exposure to phenolic compounds (treated group), there was a significant abnormal level of all the parameters as mentioned above at $P<0.05$ in comparison to normal (untreated group).

Key words : *Clarias batrachus*, Phenolic compounds, Antioxidant profile, Antioxidant enzymes

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INTRODUCTION

Phenolics are frequently considered as reactive oxygen species-generating agents leading to major cell damage, such as oxidation of membrane polyunsaturated lipids (Pradhan *et al.*, 1990). Some of them are scavengers for free radical species, while others are considered as reactive oxygen species generating agents (Wilson and Laurent, 2002). The studies showed that 3-(dimethylamino) phenol increased the level of free radicals and changed the properties of the cell membrane, caused strong oxidation of haemoglobin and also changed the activity of glutathione peroxidase, catalase, superoxide dismutase and acetylcholinesterase in human erythrocytes (Bukowska *et al.,* 2000). The possible effects of the waste water contamination of a petroleum industry on the oxidant/antioxidant status of muscle and liver tissues from fish in the Kizilirmak River, Kirikkale, Turkey (Avci *et al.*, 2005). Results obtained suggest that some contaminants from the petrochemical industry cause oxidation in fish muscle tissues by impairing the antioxidant system. In the present investigaion, antioxidant profile of *Clarias batrachus* was determined after exposure to phenolic compounds. The results reveal that the responses of antioxidant enzymes such as catalase, super oxide dismutase, glutathione peroxidase, glutathione-S-transferase and non- enzymatic antioxidant glutathione showed that the organism is experiencing severe oxidative stress which confirms that phenolic

compounds can act as potent free radical generators. Indicators of lipid peroxidation such as malondialdehyde, conjugated diene and hydroperoxide levels showed that extensive lipid peroxidation occurs on exposure to different phenolic compounds.

Justification :

Many pollutants exist in the aquatic environment, for short or long periods, at sub-lethal levels. These levels are not noticed because they do not cause immediate fish mortality. However, the consequences of such effects are morphological and physiological, causing illness and reducing fitness for life. Therefore, the simple fact that a sub-lethal concentration is considered safe because it does not kill any fish does not means that it can be used indiscriminately because contaminant effects can weaken fish, rendering them more susceptible to mortality from other causes. In many cases, alterations at the cellular or sub cellular level are not by themselves diagnostic of a particular type of pollutant. Hence, in the present investigation a combination of biochemical and histopathological studies has been adopted for determining a specific response to a particular pollutant. In the present study freshwater-adapted fish *Clarias batrachus* was exposed to different phenolic compounds at both *in vitro* and *in vivo* conditions.

RESEARCH METHODOLOGY

Phenolic compounds used for the study :

Analar monohydric phenol (C_6H_5OH, MW-94.11) and *m*-cresol ($CH_3C_6H_4OH$, MW -108.14) purchased from Sisco Research Laboratories (SRL), India were used. The nominal concentrations needed were prepared from fresh stock solutions.

Experimental design :

Collection and maintenance of test fish :

Clarias batrachus (20-25 g) were collected from fresh water source from Lucknow, U.P. region, India and brought to the National Bureau of Fish Genetic Resources (NBFGR) Laboratory, Lucknow (U.P), India in small aerated tanks. In the laboratory, they were kept in large tanks where a continuous and gentle flow of tap water was maintained. The tap water had dissolved oxygen content of 7.8 ppm, hardness below detectable amounts, pH 7.0 ± 0.37, temperature 26 ± 30^0C and salinity 0 ppt. They were fed on a commercial diet *ad libitum* and were acclimated in tanks for a month before the experiment.

Processing of tissue samples :

After the experimental period (21 days) the fishes were killed by pithing (by damaging the brain and severing the spinal cord between the head and trunk region using a sharp needle) and the tissues such as liver, gills, kidney and muscle were removed from its body, wiped thoroughly, using blotting paper to remove blood and other body fluids. Then they were washed in ice cold 0.33 M sucrose and again blotted dry and the desired amounts of the tissue were weighed and used.

Preparation of serum samples :

Blood was drawn from the common cardinal vein using 1 ml syringe. The blood collected was then kept at room temperature for 30 minutes to separate the serum. The serum thus obtained was then subjected to centrifugation at 3000 rpm for 3 minutes. The serum separated was then stored at -20°C until assayed.

Parameters investigated :

Assay of superoxide dismutase (*SOD*) (*E.C.1.15.1.1*):

Superoxide dismutase in different tissues was determined as per the method described by Kakkar *et al.* (1984). Weighed samples of tissues were homogenised in 0.33 M sucrose and subjected to differential centrifugation under cold conditions to obtain the cytosol fraction. Before estimating the activity, an initial purification was done by precipitating the protein from the supernatant with 90 per cent ammonium sulphate and this fraction was then dialysed against 0.0025 M Tris-HCl buffer (pH 7.4). The supernatant was used as the enzyme source. Assay mixture contained 1.2 ml of sodium pyrophosphate buffer, 0.1 ml of PMS, 0.3 ml of NBT, 1.3 ml of distilled water and 0.1 ml of the enzyme source. The tubes were kept at 30°C for one minute and then 0.2 ml of NADH were added and incubated at 30°C for 90 seconds and the reaction was stopped by the addition of 1 ml of glacial acetic acid. Reaction mixture was shaken vigorously with 4.0 ml of n-butanol. The mixture was allowed to stand for 10 minutes and centrifuged. The upper butanol layer was removed. Absorbance of the chromogen in butanol was measured at 560 nm against n-butanol blank. A system devoid of enzyme served as control. One unit of enzyme activity is defined as the

enzyme concentration required inhibiting chromogen production by 50 per cent in one minute under the assay conditions and specific activity was expressed as units / mg protein.

Assay of catalase (*CAT*) (*E.C.1.11.1.6*) :

Catalase level in different tissues was determined using the method as described by Maehly and Chance (1955). The estimation was done spectrophotometrically following the decrease in absorbance at 230 nm. The reaction mixture contained 0.01 M phosphate buffer, 30 mM hydrogen peroxide and the enzyme extract prepared by homogenizing the tissue in phosphate buffer and centrifuging at 5000 rpm. Specific activity was expressed as international units / mg protein. 1 1U = change in absorbance / min/ extinction co-efficient (0.021).

Assay of glutathione peroxidase (*GPx*) (*E.C. 1.11.1.9*):

Glutathione peroxidase in different tissues was estimated by the method as described by Rotruck *et al.* (1973). Weighed samples of different tissues were homogenized in a known volume of Tris buffer. To 0.2 ml of Tris buffer, 0.2 ml EDTA, 0.1 ml sodium azide and 0.5 ml tissue homogenate were added and mixed well. To this mixture 0.2ml of GSH followed by 0.1 ml H_2O_2 solution were added. The contents were mixed and incubated at 37°C for 10 minutes along with a control containing all reagents except tissue homogenate. After 10 minutes the reaction was arrested by the addition of 0.5 ml of 10 per cent TCA. Tubes were centrifuged and the supernatant was assayed for GSH. The values were expressed as µg of GSH / min/ mg protein.

Assay of glutathione-S-transferase (*GST*) (*E.C.2.5.1.18*) :

Glutathione-S-transferase in different tissue was determined using the method as described by Beutler (1986). All the tissues were homogenized in 0.5 M phosphate buffer. The reaction mixture contained 200µl phosphate buffer, 20µl CDNB and 680µl distilled water. The tubes were incubated at 37°C for 10 minutes and added 50µl of GSH. After mixing well, added 50µl of tissue extract to the tube. Increase in absorbance was noted at 340nm for 5 minutes in a UV-visible spectrophotometer. Values were expressed in µmoles of CDNB complexed / min/ mg protein. The extinction co-efficient between CDNB -GSH conjugate is 9.6 mM^{-1} cm^{-1}.

Estimation of total reduced glutathione (*GSH*) :

Total reduced glutathione was estimated by the method as described by Elia *et al*. (2003). Precipitated protein in the homogenates of gills, liver, kidney and muscle with 0.1 ml 5 per cent TCA and 0.4 ml distilled water. Mixed the contents well for complete precipitation of proteins and centrifuged. To 0.5 ml clear supernatant, added 2.5 ml of 0.2 M phosphate buffer and 50 µl of DTNB. Read the absorbance at 412 nm against a blank containing all the reagents. A series of standards were run along with blank treated in a similar manner to determine the glutathione content. Values were expressed as nmoles/100 g wet tissue.

Estimation of conjugated dienes (*CD*) :

The concentration of conjugated dienes was estimated according to the method as described by Retnagol and Ghoshal (1966). Membrane lipids were extracted and evaporated to dryness as described for the iodometric assay for hydroperoxides. The lipid residue was dissolved in 1.5 ml of cyclohexane and the absorbance at 233 nm was determined against a cyclohexane blank. Molar extinction co-efficient of conjugated dienes is 2.52 x 104 M^{-1} cm^{-1}.

Estimation of hydroperoxides (*HP*) :

Hydroperoxides was estimated by method as described by Mair and Hall (1977). 1ml of the tissue homogenate of the different tissues was mixed thoroughly with 5 ml of chloroform: methanol (2:1) followed by centrifugation at 1000×g for 5 minutes to separate the phases. 3 ml of the lower chloroform layer was recovered using a syringe and placed in a test tube and dried in a 45°C water bath. 1 ml of acetic acid: chloroform (3:2) mixture followed by 0.05 ml of potassium iodide was quickly added and the test tubes were stoppered and mixed. The tubes were placed in the dark at room temperature for exactly 5 minutes followed by the addition of 3 ml of cadmium acetate. The solution was mixed and centrifuged at 1000 × g for 10 minutes. The absorbance of the upper phase was read at 353 nm against a blank containing the complete assay mixture except the tissue homogenate. Molar extinction co-efficient of hydroperoxide is 1.73×104 M^{-1} cm^{-1}.

Estimation of malondialdehyde :

Malondialdehyde was estimated by the method as

described by Niehaus and Samuelson (1968). The tissue homogenate of different tissues were prepared in Tris-HCI buffer and was combined with thiobarbituric acid reagent and mixed thoroughly and heated for 15 minutes in a boiling water bath. It was then cooled and centrifuged for 10 minutes at 600×g. The absorbance of the sample was read spectrophotometrically at 535 nm against a reagent blank that contained no tissue extract. The extinction co-efficient for malondialdehyde is 1.56 ×105 M^{-1} cm^{-1}. The values were expressed as mill moles / 100g wet wt of tissue.

RESEARCH FINDINGS AND ANALYSIS

In the present investigation, it was found that different antioxidant enzymes gets abnormally decreased leading to the malfunctioning of organs and tissues of *Clarias batrachus*. The results thus, show the abnormal and lethargic effect of phenolic compounds as pollutants/ xenobiotics on antioxidant profile of *Clarias batrachus*. The results of each of the biomarker assessed are as follows:

Superoxide dismutase (SOD) :

Two-factor ANOVA followed by Tukey's test showed that there was significant (P<0.05), (Table 1) variation in SOD activity between treatments and also between tissues. Between the treated groups, both phenol and *m*-cresol treated groups showed significant variation in SOD activity and also with the control. SOD activity was found to be significantly (P<0.05) elevated in gills, liver and kidney of *Clarias batrachus* treated with phenol compared to control and among these tissues liver showed the maximum activity, whereas the fishes treated with *m*-cresol showed significantly elevated activity in liver, kidney and muscle compared to control. A significantly (P<0.05) decreased activity compared to control was shown by gills treated with *m*-cresol and muscle treated with phenol.

Table 1 : Effect of different phenolic compounds on superoxide dismutase activity in *Clarias batrachus*

Tissue	SOD activity (U/mg protein)		
	Control	Phenol	*m*-cresol
Gills	$_{b}12.76\pm2.73^{A}$	$_{c}23.32\pm2.76^{B}$	$_{a}05.61\pm2.83^{B}$
Liver	$_{b}12.70\pm1.10^{C}$	$_{c}27.56\pm1.10^{D}$	$_{a}15.67\pm1.10^{D}$
Kidney	$_{b}13.53\pm0.25^{C}$	$_{c}17.28\pm0.27^{C}$	$_{a}15.67\pm0.28^{D}$
Muscles	$_{b}03.06\pm0.24^{A}$	$_{c}02.09\pm0.27^{B}$	$_{a}04.12\pm0.28^{C}$

Values in the same column with different upper case letters vary significantly (P<0.05) between tissues and values in the same row with different lower case letters vary significantly (P<0.05) between treatment groups. Values are expressed as units/mg protein. One unit is defined as the amount of enzyme which gives 50 per cent inhibition of formazon formation / minute. Each value represents the mean ± S.D of three separate experiments.

Estimation of catalase (CAT) :

In the present study catalase activity in different tissues of *Clarias batrachus* treated with different phenolic compounds showed significant variations (P <0.05), (Table 2) compared to control group. Turkey's test showed significant difference between phenolic compounds treated groups and also with the control. Highest CAT activity was found in the liver of fishes treated with *m*-cresol. On treatment with both phenol and *m*-cresol gills, liver and kidney showed significantly elevated CAT activity compared to control. Comparison between groups treated with different phenolic compounds revealed that there was significant increase (P<0.05) in CAT activity in all tissues compared to control except in muscle. Muscle showed a statistically significant decreased activity compared to control.

Table 2 : Effect of different phenolic compounds on catalase activity in *Clarias batrachus*

Tissue	CAT activity (IU/mg)		
	Control	Phenol	*m*-cresol
Gills	$_{a}11.76\pm2.73^{B}$	$_{b}15.32\pm2.76^{B}$	$_{c}14.61\pm2.83^{C}$
Liver	$_{a}11.54\pm1.10^{C}$	$_{b}34.56\pm1.10^{D}$	$_{c}38.67\pm1.10^{D}$
Kidney	$_{a}4.82\pm0.25^{C}$	$_{b}08.28\pm0.27^{C}$	$_{c}09.67\pm0.28^{D}$
Muscles	$_{a}3.05\pm0.24^{A}$	$_{b}02.09\pm0.27^{B}$	$_{c}02.12\pm0.28^{B}$

Values in the same column with different upper case letters vary significantly (P<0.05) between tissues and values in the same row with different lower case letters vary significantly (P<0.05) between treatment groups. One IU = Change in absorbance at 230 nm / min, extinction co-efficient = 0.021. Each value represents the mean ± S.D of three separate experiments.

Estimation of glutathione peroxidase (GPx) :

Glutathione peroxidase activity showed an overall significant change (P<0.05) in experimental groups of animal (Table 3) compared to control. Turkey's test showed significant difference between phenolic

compounds treated groups and also with the control. Statistical analysis between tissues showed that GPx activity was found to show a statistically significant (P<0.05) decreased activity in liver and kidney of the treated groups compared to control. Whereas gills treated with phenol showed a decreased GPx activity compared to control. On treatment with both phenol and *m*-cresol muscle showed a significantly (P<0.05) elevated activity compared to control.

Table 3 : Effect of different phenolic compounds on glutathione peroxidase activity in *Clarias batrachus*

Tissue	GPx activity (µg GPx/min./mg protein)		
	Control	Phenol	*m*-cresol
Gills	$_{c}11.76\pm2.73^{B}$	$_{b}06.32\pm2.76^{B}$	$_{a}15.61\pm2.83^{C}$
Liver	$_{c}10.54\pm1.10^{C}$	$_{b}03.56\pm1.10^{B}$	$_{a}03.67\pm1.10^{A}$
Kidney	$_{c}12.82\pm0.25^{C}$	$_{b}04.28\pm0.27^{B}$	$_{a}08.67\pm0.28^{B}$
Muscles	$_{c}5.05\pm0.24^{A}$	$_{b}07.09\pm0.27^{B}$	$_{a}08.12\pm0.28^{C}$

Values in the same column with different upper case letters vary significantly (P<0.05) between tissues and values in the same row with different lower case letters vary significantly (P<0.05) between treatment groups. Values are expressed as µg of GPx/min/mg protein. Each value represents the mean ± S.D. of three separate experiments.

Estimation of glutathione-S-transferase (GST) :

In the present study, glutathione-S-transferase activity in different tissues of *Clarias batrachus* treated with different phenolic compounds showed significant variations (P<0.05), (Table 4) compared to control group. Turkey's test showed significant difference among the phenolic compounds treated groups and also with the control. Among the tissues treated with different phenolic compounds highest GST activity was seen in liver. Both kidney and muscle showed significantly (P<0.05) decreased GST activity compared to control. Significant differences were found in GST activity between the phenol and *m*-cresol treated groups and also with the control.

Table 4 : Effect of different phenolic compounds on glutathione S-transferase activity in *Clarias batrachus*

Tissue	Glutathione S- transferase activity (µg GST/min./mg protein)		
	Control	Phenol	*m*-cresol
Gills	$_{b}27.76\pm2.73^{B}$	$_{c}30.32\pm2.76^{C}$	$_{a}22.61\pm2.83^{C}$
Liver	$_{b}36.54\pm1.10^{C}$	$_{c}48.56\pm1.10^{D}$	$_{a}70.67\pm1.10^{D}$
Kidney	$_{b}21.82\pm0.25^{C}$	$_{c}09.28\pm0.27^{B}$	$_{a}14.67\pm0.28^{A}$
Muscles	$_{b}07.23\pm0.24^{C}$	$_{c}04.09\pm0.27^{B}$	$_{a}1.21\pm0.28^{B}$

Values in the same column with different upper case letters vary significantly (P<0.05) between tissues and values in the same row with different lower case letters vary significantly (P<0.05) between treatment groups. Values are expressed in µmoles of CDNB complexed / min/ mg protein. Each value represents the mean ± S.D of three separate experiments.

Two-factor ANOVA followed by Turkey's test showed that there was significant (P<0.05) (Table 5) variation in total reduced glutathione content between treated groups and between tissues treated with different phenolic compounds. There was statistically significant (P<0.05) different changes in the GSH level among the treated groups and between the treated groups and the control. Among the tissues, gills, liver and muscle showed significantly (P<0.05) elevated activity compared to control but the kidney in both the treated groups showed statistically significant (P<0.05) reduced activity compared to control.

Table 5 : Effect of different phenolic compounds on total reduced glutathione activity in *Clarias batrachus*

Tissue	Total activity reduced glutathione (GSH) (nmoles/100 g wet tissue)		
	Control	Phenol	*m*-cresol
Gills	$_{a}1657.76\pm2.73^{B}$	$_{b}2406.23\pm2.76^{C}$	$_{c}2289.61\pm2.83^{C}$
Liver	$_{a}1484.54\pm1.10^{C}$	$_{b}2745.76\pm1.10^{C}$	$_{c}1789.67\pm1.10^{D}$
Kidney	$_{a}1112.34\pm0.25^{C}$	$_{b}983.28\pm0.27^{B}$	$_{c}814.67\pm0.28^{B}$
Muscles	$_{a}756.23\pm0.24^{A}$	$_{b}756.09\pm0.27^{A}$	$_{c}712.21\pm0.28^{B}$

Values in the same column with different upper case letters vary significantly (P<0.05) between tissues and values in the same row with different lower case letters vary significantly (P<0.05) between treatment groups. Values were expressed as n moles/100 g wet tissue. Each value represents the mean ± S.D of three separate experiments.

Estimation of conjugated dienes (CD) :

Conjugated diene level in all the phenolic compounds treated groups was significantly (Table 6) (P<0.05) different when compared to control. Among the tissues gills, liver and muscle showed a statistically significant elevated CD level in both the treated groups compared to control whereas the kidney in both the treated groups showed a statistically significant (P<0.05) reduced level compared to control.

Table 6 : Effect of different phenolic compounds on conjugated dienes in *Clarias batrachus*

Tissue	Conjugated dienes concentration (mmoles/100g wet tissue)		
	Control	Phenol	*m*-cresol
Gills	$_{a}30\pm2.73^{A}$	$_{b}45.23\pm2.76^{B}$	$_{c}45.61\pm2.83^{B}$
Liver	$_{a}32\pm1.10^{C}$	$_{b}50.76\pm1.10^{D}$	$_{c}48.67\pm1.10^{D}$
Kidney	$_{a}10\pm0.25^{C}$	$_{b}9.28\pm0.27^{B}$	$_{c}8.67\pm0.28^{B}$
Muscles	$_{a}20\pm0.24^{A}$	$_{b}18.09\pm0.27^{B}$	$_{c}24.21\pm0.28^{C}$

Values in the same column with different upper case letters vary significantly (P<0.05) between tissues and values in the same row with different lower case letters vary significantly (P<0.05) between treatment groups. Values were expressed as mmoles/100g wet tissue. Each value represents the mean ± S.D of three separate experiments.

Estimation of hydroperoxides (HP) :

The level of hydroperoxides in the groups treated with both the phenol and *m*-cresol showed statistically significant (P<0.05) (Table 7) difference between them and also with the control group. Tissues such as gills, liver, kidney and muscle showed statistically significant (P<0.05) elevated levels compared to control. Among the tissues the highest level of hydroperoxide was seen in liver.

Table 7 : Effect of different phenolic compounds on hydroperoxides in *Clarias batrachus*

Tissue	Hydroperoxides (HP) (mmoles/100 g wet tissue)		
	Control	Phenol	m-cresol
Gills	$_{a}13.27\pm2.73^{A}$	$_{b}15.23\pm2.76^{B}$	$_{c}7.61\pm2.83^{C}$
Liver	$_{a}28\pm1.10^{C}$	$_{b}38.76\pm1.10^{D}$	$_{c}33.67\pm1.10^{D}$
Kidney	$_{a}14\pm0.25^{C}$	$_{b}17.28\pm0.27^{C}$	$_{c}15.67\pm0.28^{D}$
Muscles	$_{a}08\pm0.24^{B}$	$_{b}11.09\pm0.27^{A}$	$_{c}12.21\pm0.28^{A}$

Values in the same column with different upper case letters vary significantly (P<0.05) between tissues and values in the same row with different lower case letters vary significantly (P<0.05) between treatment groups. Values were expressed as mmoles/100g wet tissue. Each value represents the mean ± S.D of three separate experiments.

Estimation of malondialdehyde (MDA) :

No significant difference (Table 8) in MDA level was found in gills, kidney and muscle among the treated groups. Among the tissues, statistically significant (P<0.05) elevated MDA level was found in gills and liver compared to control.

Table 8 : Effect of different phenolic compounds on MDA level in *Clarias batrachus*

Tissue	MDA level (mmoles/100 g wet tissue)		
	Control	Phenol	*m*-cresol
Gills	$_{a}0.081\pm2.73^{B}$	$_{b}0.095\pm2.76^{B}$	$_{b}0.09\pm2.83^{C}$
Liver	$_{a}0.142\pm1.10^{C}$	$_{b}0.158\pm1.10^{D}$	$_{b}0.161\pm1.10^{D}$
Kidney	$_{a}0.032\pm0.25^{C}$	$_{b}0.03\pm0.27^{B}$	$_{b}0.03\pm0.28^{A}$
Muscles	$_{a}0.052\pm0.24^{A}$	$_{b}0.05\pm0.27^{B}$	$_{b}0.05\pm0.28^{C}$

Values in the same column with different upper case letters vary significantly (P<0.05) between tissues and values in the same row with different lower case letters vary significantly (P<0.05) between treatment groups. Values were expressed as m moles/100g wet tissue. Each value represents the mean ± S.D. of three separate experiments.

The antioxidant defense mechanism of *Clarias batrachus* was responsive to the exposure of different phenolics. In the present study, almost all the tissues treated with phenol and m-cresol for 21 days in *Clarias batrachus* showed significantly elevated SOD and CAT activity compared to control. SOD is the first enzyme to respond against oxygen radicals (McCord and Fridovich, 1969) and is the one that offers the greatest response to oxidative stress (Winston *et al.*, 1996). The tissue specific increase in SOD activity showed the following trend for fishes treated with phenol: kidney > gills > liver whereas the muscle showed a significantly decreased SOD activity compared to control. On treatment with m-cresol, tissues such as liver, kidney and muscle showed a significantly elevated activity whereas gills showed a significantly decreased activity compared to control. Changes in the levels of superoxide dismutase have been detected in fishes exposed to various degrees of oxygen tension (Lushchak *et al.*, 2001) and environmental perturbations (Achuba and Osakwe, 2003). Superoxide dismutase is inducible in mammals and micro-organisms and the level of the enzyme increases with an increased need of protection against toxic oxygen radicals (Fridovich, 1974 and Trostler *et al.*, 1979). Mn-containing superoxide dismutase and Cu/Zn dependent superoxide dismutase are involved in the general defense system against natural or chemically induced production of reactive oxygen species (Fridovich, 1986). Catechol increases the reduction of O_2 and this may have resulted in an increased

SOD activity. Also catechol reduces the dismutation of O_2, and thus, leads to the production of larger amounts of H_2O_2. Thus, for the detoxification of increased H_2O_2 generated a significantly elevated CAT activity was observed in gills, liver and kidney of fishes treated with both the phenolics whereas muscle showed a significantly decreased CAT activity compared to control in both phenol and m-cresol treated groups. An increased generation of H_2O_2 may have occurred due to several reasons such as oxygen depletion (Penning *et al.*, 1996), dismutation reaction of O^2- catalyzed by increased SOD activity. The elevated CAT activity observed may be for the detoxification of increased H_2O_2 formed from different reactions. Therefore, the SOD-CAT system provides the first defence against oxygen toxicity. Perhaps a paroxysmal proliferation may have also occurred as they are cell organelles that play key roles in multiple cell functions especially in the metabolism of ROS. The most abundant peroxisomal enzyme is CAT and the proliferation may have resulted in elevated CAT activity. Increase of SOD and CAT in liver is reported in some fish species under oxidative stress (Sayeed *et al.*, 2003; Gull *et al*., 2004; Zhang *et al.*, 2004; Nam *et al*., 2005; Wilhelm-Filho *et al*., 2005). Considering the results for each tissue in both treated groups, it was found that liver showed the highest SOD and CAT antioxidant activity, both enzymes appearing to have an important role in to be highly elevated in liver on exposure to phenolics, since liver plays an important role in the detoxification of xenobiotics and in elimination by conjugating them with glutathione. GST-mediated conjugation may be an important mechanism for detoxifying peroxidised lipid breakdown products, which have a number of adverse biological effects when present in high amounts. Induced GST activity indicates the role of this enzyme in protection against the toxicity of xenobiotic-induced lipid peroxidation. Many studies analyzing GST in liver of fish exposed to different insecticides showed an enzymatic induction. However, inhibition of GST activity has also been reported in gills of mosquito fish exposed to carbofuran (Rondon-von Osten *et al.*, 2005). Thus, it is possible that the enzyme is regulated *in vivo* by, for instance, thiol-disulphide interchange and proteolysis or by some other mechanism. Since reactive metabolites of foreign compounds are substrates for glutathione transferase, an attractive idea would be that these metabolites modify the microsomal glutathione transferase covalently, thereby increasing the enzyme activity by which these reactive metabolites are eliminated through conjugation. This would allow the cell to adjust rapidly to exposure to reactive compounds. The microsomal metabolism of phenol to species which will bind to proteins is most likely catalyzed by P450 monooxygenase. These enzymes are probably the major targets for the covalent binding of phenol. It is likely that the electrophilic metabolites benzoquinone and 2- hydroxyl benzoquinone conjugate with the sulphydryl group of the enzyme, thereby activating the enzyme. In summary, microsomal glutathione transferase can be activated by reactive metabolites of phenol and *m*-cresol, and is caused by covalent binding of the metabolites to the enzyme. GSH is the major cytosolic low molecular weight sulphydryl compound that acts as a cellular reducing and a protective reagent against numerous toxic substances including most inorganic pollutants, through the -SH group. Gills, combating the sequential generation of superoxide radical (O^{2-}) and hydrogen peroxide (H_2O_2) from the intense metabolic activity characteristic of this tissue. The significant increase in catalase and superoxide dismutase activities in gills, liver and kidney examined may represent an adaptive response to protect the fish from free radical toxicity induced by phenolic compounds. GPx glutathione peroxidase activity, a seleno-enzyme that neutralizes ROS such as organic and hydrogen peroxides activity in gills, liver and kidney of fishes treated with phenol and m-cresol showed a significantly decreased activity compared to control. Whereas muscle in both treated groups showed a significantly enhanced activity compared to control. GPx activities are fundamental to remove hydrogen peroxide from cytoplasm, however, only the GPx activity was decreased in *C. batrachus* exposed to both the phenolics. In theory, reduced enzymatic activity implies that some ROS are not being quenched, thus, predisposing cells to oxidative stress. The low GPx activity might be due to a direct phenol inhibition of enzyme synthesis or due to increased generation of hydroperoxide which may have inhibited the enzyme activity. Also catechol toxicity is mainly associated with damage to the protein and generation of hydrogen peroxide, which is capable of causing further damage (Barreto *et al.*, 2009). Significantly elevated GPx activity in muscle shows that an induction in glutathione peroxidase activity has occurred in this tissue. GST is a multicomponent enzyme involved in the detoxification of many xenobiotics, which plays an important role in protecting tissues from oxidative stress. GST was found to be strongly inhibited in kidney

and muscle on exposure to different phenolic compounds. GST activity was found liver and muscle showed elevated GSH level when treated with phenolics. Among the tissues, GSH level was found to be highest in liver compared to other tissues which may be due to an adaptive mechanism to slight oxidative stress through an increase in its synthesis which can be provided for the increased GST activity. However, a depletion of GSH was observed in kidney which shows that severe oxidative stress may suppress GSH levels due to loss of adaptive mechanisms and the oxidation of GSH to GSSG. During scavenging the ROS, GSH is oxidized and forms glutathione-protein mixed disulphides; hence, the cell's ability to reduce or synthesize GSH is the key to how effectively the cell can manage the oxidative stress. Total glutathione will be a prospective biological index to indicate exposure to contaminants. Due to its function in resisting the reactive oxygen toxicity, the changing degree for total glutathione can serve as markers of exposure to pollutants which disturb the piscine oxyradicals. The conjugated diene level was found to be elevated in liver, kidney and muscle of both the treated groups and also in gills treated with phenol. CD is the initial peroxidative product and is an accurate indicator of lipid peroxidation and its elevated level indicated that lipid peroxidation has been initiated. An increased hydroperoxide level was observed in liver, kidney and muscle of both the treated groups which may be due to decreased GPx activity observed in these tissues. This may be because GPx catalyzes the reduction of H_2O_2 derived from oxidative metabolism as well as peroxides from oxidation of lipids and is considered the most effective enzyme against lipid peroxidation. Being more polar than parent lipids, hydroperoxides perturb membrane structure/function and can be deleterious to cells. An increased MDA level was observed in both gills and liver on exposure to different phenolics indicating that elevated antioxidant enzyme activities were not efficient enough to prevent lipid peroxidation in these tissues. Significant oxidative damage and lipid peroxidation should theoretically occur if antioxidant defenses were overwhelmed by ROS production. In addition to changes in the antioxidant defense system, one of the hallmarks of oxidative stress is damage to biological macromolecules such as the phospholipids of cell membranes. MDA is a major oxidation product of peroxidized polyunsaturated fatty acids and increased MDA content is an important indicator of lipid peroxidation. Taken as a whole, our data seems to implicate phenolic compounds as a potent mediator of free radical generation in fish.

Conclusion :

Indicators of lipid peroxidation such as malondialdehyde, conjugated diene and hydroperoxide levels show that extensive lipid peroxidation occurs on exposure to different phenolic compounds. Branchial functioning was impaired and hence the ionic homeostasis in *Clarias batrachus* was affected on exposure to sub-lethal concentrations of both phenol and *m*-cresol. Phenol and *m*-cresol is giving negative impact on enzymes involved in important metabolic pathways as their activity is disturbed such as impaired food intake, the increased energy cost of homeostasis, tissue repair and the detoxification mechanism during stress. Even the expression of antioxidant enzymes was greatly affected on exposure to phenolic compounds such as damage to the microsomal membrane as these enzymes are localized exclusively in the membranes of the endoplasmic reticulum.

LITERATURE CITED

Achuba, F.I. and Osakwe, S.A. (2003). Petroleum-induced free radical toxicity in African cat fish (Clarias gariepinus) Fish. *Physiol. Biochem.,* **29** (2) : 97-103.

Avci, A., Kacmaz, M. and Durak, I. (2005). Peroxidation in muscle and liver tissues from fish in a contaminated river due to a petroleum refinery industry. *Ecotoxicol. Environ. Saf.,* **60** (1) : 101-105.

Barreto, G., Madureira, D., Capani, F., Aon-Bertolino, L., Saraceno, E. and Alvarez-Giraldez, L.D. (2009). The role of catechols and free radicals in benzene toxicity: An oxidative DNA damage pathway. *Environ. & Mol. Mutagenesis*, **50** (9) : 771-780.

Beutler, E. (1986). *Red cell metabolism*. Churchill Livingstone Pub, New York 70 pp.

Bukowska, B., Chajdys, A., Duda, W. and Piotr, D. (2000). Catalase activity in human erythrocytes: effect of phenoxyherbicides and their metabolites. *Cell Biol. Internat.,* **24** (10) : 705-711.

Elia, A.C., Galarini, R., Taticchi, M.I., D¨orr, A.J.M. and Mantilacci, L. (2003). Antioxidant responses and bioaccumulation in Ictalurus melas under mercury exposure. *Ecotoxicol. Environ. Saf.,* **55** (2) : 162-167.

Fridovich, I. (1974). Superoxide dismutase. In: Meister. *Advances in enzymology*. Vol. 41 New York: John Wiley & Sons. Inc. pp.35-97.

Fridovich, I. (1986). Biological effects of the superoxide radical. *Arch. Biochem. Biophys.,* **247** (1) : 1-11.

Gull, S., Belge-Kurutas, E., Yildiz, E., Sahan, A. and Doran, F. (2004). Pollution correlated modifications of liver antioxidant system and histopathology of (Cyprinidae) living in Seyhan Dam Lake, Turkey. *Environ. Int.,* **30** (5) : 605-609.

Kakkar, P., Das, B. and Viswanathan, D.N. (1984). A modified spectrometric assay of superoxide dismutase. *Indian. J. Biochem. Biophys.,* **21** (2) : 130-132.

Lushchak, V.I., Lushchak, L.P., Mota, A.A. and Hermes-Lima, M. (2001). Oxidative stress and antioxidant defences in goldfish *Carassius auratus* during anoxia and reoxygenation. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.,* **280** (1) : 100-107.

Maehly, A.C. and Chance, B. (1955). Assay of catalases and peroxidases. In: *Methods in enzymology*, vol II, (Ed. S. P. Colowick and N.O. Kaplan) Academic Press, New York, London. 764pp.

Mair, R.D. and Hall, T. (1977). Inorganic peroxides (Ed. D.E. Swenn and Wintky). 532. N4. Intersciences 2.

McCord, J.M. and Fridovich, I. (1969). Superoxide dismutase. An enzymatic function for erythrocuprein (Heamocuprein). *J. Biol. Chem.,* **244** (22) : 6049-6055.

Nam, Y., Cho, Y., Choi, B., Kim, K., Kim, S. and Kim, D. (2005). Alteration of oxidative enzyme at the mRNA level during short-term starvation of therockbream, Oplegnatus fasciatus. *Fisheries Sci.,* **71** : 1385-1387.

Niehaus, W.G. Jr and Samuelson, B. (1968). Formation of malonaldialdehyde from phospholipids arachidonate during microsomal lipid peroxidation. *Eur. J. Biochem.,* **6** (1) : 126-130.

Penning, T.M., Ohnishi, S.T., Ohnishi, T. and Harvey, R.G. (1996). Generation of reactive oxygen species during the enzymatic oxidation of polycyclic aromatic hydrocarbon trans-dihydrodiols catalyzed by dihydrodiol dehydrogenase. *Chem. Res. Toxicol.,* **9** (1) : 84-92.

Pradhan, D., Weiser, M., Lumley-Sapanski, K., Frazier, D., Kemper, S., Williamson, P. and Schlegel, R.A. (1990). Peroxidation-induced perturbations of erythrocyte lipid organization. *Biochem. Biophys. Acta.,* **1023** (3) : 398-404.

Retnagol, R.O. and Ghoshal, A.K. (1966). Quantitative estimation of peroxidative decomposition of rat liver microsomal and mitochondrial lipids after carbon tetrachloride poisoning. *Exp. & Mole. Path.,* **5** : 413-426.

Rondon-von Osten, J., Ortiz-Arana, A., Guilhermino, L. and Soares, A.M.V.M. (2005). *In-vivo* evaluation of three biomarkers in the mosquito fish (*Gambusia yucatana*) exposed to pesticides. *Chemosphere.* **58** (5) : 627-636.

Rotruck, J.J., Pope, A.L., Ganthe, H.E., Swanson, A.B., Hafeman, D.G. and Hoekstra, W.G. (1973). Selenium: biochemical role as a component of glutathione peroxidase. *Sci.,* **179** (4073) : 588-590.

Sayeed, I., Parvez, S., Pandey, S., Bin-Hafeez, B., Haque, R. and Raisuddin, S. (2003). Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, Channa punctatus Bloch. *Ecotoxicol. Environ. Saf.,* **56** (2) : 295-301.

Trostler, N., Brady, P.S., Romas, D.R. and Leveille, G.A. (1979). Influence of dietary vitamin E on malondialdehyde levels in liver and adipose tissue and on glutathione peroxidase and reductase activities in liver and erythrocytes of lean and obese mice. *J. Nutr.,* **109** (2) : 345-352.

Wilhelm-Filho, D., Torres, M.A., Zanibonifilho, E. and Pedrosa, R.C. (2005). Effect of different oxygen tensions on weight gain, feed conversion, and antioxidant status in piapara, *Leporinus elongatus* (Valenciennes, 1847). *Aquacul.,* **244** (1-4) : 349-357.

Wilson, J.M. and Laurent, P. (2002). Fish gill morphology: inside out. *J. Exp. Zool.,* **293** (3) : 192-213.

Winston, G.W., Moore, M.N., Kirchin, M.A. and Soverchia, C. (1996). Production of reactive oxygen species (ROS) by haemocytes from the marine mussel, Mytilus edulis. *Comp. Biochem. Physiol.,* **113** (2) : 221-229.

Zhang, J., Shen, H., Wang, X., Wu, J. and Xue, Y. (2004). Effects of chronic exposure of 2, 4-dichlorophenol on the antioxidant system in liver of freshwater fish *Carassius auratus*. *Chemosphere,* **55** (2) : 167-174.