ASIAN JOURNAL OF BIOMEDICAL & PHARMACEUTICAL SCIENCES

e-ISSN: 2249-622X

RESEARCH ARTICLE

Evaluation of Changes in Metabolic parameters and Enzymes Involved in Metabolic Pathways in *Clarias batrachus* after Exposure to Phenolic Compounds

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Received:
13th May 2013
Received in revised form:
30th June 2013
Accepted:
4th July 2013
Available online:
10th July 2013

OPEN ACCESS

Online ISSN 2249–622X
http://www.jbiopharm.com

ABSTRACT

Water pollution is significant only when it influences living or biological systems either directly or indirectly. In a broad sense, it can be depicted as a normal consequence of the growth of organisms including man in or near the aquatic habitat. The presence of toxic pollutants in aquatic ecosystems poses a serious threat to environmental health. Industrialization and growth of human population have led to a progressive deterioration in the quality of the earth's environment. These harmful chemicals/xenobiotics are harming the aquatic ecosystem involving flora and fauna of the water bodies. The present study is thus investigating the effect of different types of phenolic compounds on biochemical parameters and enzymes involved in metabolic pathways of *Clarias batrachus*, a fresh water fish. The present study reveals the disaster and lethargic effect of phenolic compounds on the metabolic and physical parameters of *C. batrachus*. The calculated LC50 value for phenol and m-cresol exposure in *C. batrachus* over periods of up to 96 hours is 28.45 mg l-1 and 25.7 mg l-1 respectively. The studies showed that in fishes after exposure to phenolic compounds (treated group), there was a significant decreased level of serum cortisol, total carbohydrate concentration, blood glucose concentration, pyruvate concentration and different enzymes studied which are associated with the normal functioning of metabolic organs at $P<0.05$ in comparison to normal (untreated group).

Keywords: *Clarias batrachus*, phenolic compounds, biochemical parameters, metabolic enzymes.

1. INTRODUCTION:

The unique physical and chemical properties of water have allowed life to evolve in it. The quote illustrates this point of view: "Life originated in water, is thriving in water, water being its solvent and medium, it is the matrix of life" [1]. Water pollution is significant only when it influences living or biological systems either directly or indirectly. The presence of toxic pollutants in aquatic ecosystems poses a serious threat to environmental health. Industrialization and growth of human population have led to a progressive deterioration in the quality of the earth's environment. Urban, agricultural and industrial activities release xenobiotic compounds that may pollute the aquatic habitat. About 300 million tons of synthetic compounds seep annually into water systems (rivers, lakes and sea). 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 [2-6]. Phenolic compounds decomposition in water bodies can cause toxicity, with bioaccumulation effects in animals and plants [7]. Their inhalation and ingestion may be dangerous for human health; causing systemic damage to the nervous system [8]. Quantification of phenols in water has become increasingly important because of their toxicity for humans and aquatic organisms [9]. Biomarkers are defined as changes in biological responses (ranging from molecular through cellular and physiological responses to behavioural responses) which can be related to exposure to or toxic effects of environmental chemicals [10]. Since the interaction between toxicants and biomolecules is the first step in the generation of toxic effects (preceding cellular and systemic dysfunction),the understanding of

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biochemical alterations induced by the exposure to pollutants may contribute to the prediction of toxic effects that may occur later at higher levels of biological organization. Moreover, the use of biochemical biomarkers may allow early interventions with the objective of protecting wild populations exposed to chemical agents [11]. The present study is thus stating the effect of phenolic compounds on biomarkers/biochemical parameters and metabolic enzymes in *Clarias batrachus*.

2. MATERIALS AND METHODS

2.1 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.

2.1.1 Chemicals and Reagents used for the study

The chemicals and reagents used for the study were of Analytical Grade and were procured from Sisco Research Laboratories, Ranchem and CDH, India. The diagnostic kits used in the study for biochemical testing were from Span Diagnostics Ltd. Gujrat, India.

2.2 Experimental design

(i) Collection and maintenance of test fish

Clarias batrachus (20-25g) were collected from the culture ponds of Ganga River in Allahabad (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 ± 3^0C and salinity 0 ppt (parts per thousand). They were fed on a commercial diet *ad libitum* and were acclimated in tanks for a month before the experiment.

(ii) Experimental design for lethal toxicity study

LC_{50} determination was carried out by following semi-static acute toxicity test. For the experiment, 6 fishes were transferred to large experimental tubs, each containing 18 litres of dechlorinated tap water. Eight phenol concentrations from 27 mg l^{-1} (no mortality) to 34 mg l^{-1} (100 % mortality) were chosen for the final 96-hour test to determine the 50 % lethal concentration (LC_{50}). For m-cresol eight concentrations from 19 mg l^{-1} (no mortality) to 26 mg l^{-1} (100 % mortality) were chosen for the final 96-h test to determine the 50 % lethal concentration (LC_{50}). Fishes transferred to tanks containing no toxicants served as control. Water in the control tanks and water and toxicant in the experimental tanks were renewed daily to remove the debris, taking care to give minimum disturbance to the fish. The fishes were not fed during the entire exposure period. Fishes were checked for mortality at every 24 hours interval. The LC_{50} levels and 95% confidence limits were calculated using Probit analysis (Finney, 1971). The lethal toxicity experiments were repeated wherever necessary.

(iii) Experimental design for sub-lethal toxicity studies

For conducting the biochemical study, fishes were taken in two separate tanks which contained desired concentrations of toxin, $1/10^{th}$ of LC_{50} value of phenol and m-cresol. Six replicates were kept for each experiment. The experimental fishes were dosed for 21 days. Daily the contents in the tanks were replaced with the same concentrations of toxicant so as to avoid any possible degradation of constituents of toxicant. During the experimental period of 21 days the fishes were fed on the same diet so as to avoid the effects of starvation on normal physiological processes. Any other factors likely to influence the toxicity were nullified by maintaining suitable controls in tanks that contained no toxicant.

2.3 Preparation of tissue samples for the study

After the experimental period (21days) 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.

2.4 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.

2.5 Parameters Investigated

2.5.1 Estimation of serum cortisol

The level of serum cortisol was estimated by electro-chemiluminescence immunoassay (ECLIA). To 20µl of serum sample, cortisol-specific biotinylated and a ruthenium complex labelled cortisol derivative were added. It was incubated at 37°C for 9 minutes. Streptavidin coated microparticles were added and were incubated at 37°C for 9 minutes. This forms complex which gets bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture was then aspirated into the measuring cell where the microparticles were magnetically captured onto the surface of the electrode. Unbound substances were then removed with ProCell. Voltage was applied to the electrode which induced chemiluminescent emission. It was measured by a photomultiplier. Results thus obtained were determined via a calibration curve which was

instrument specific generated by a 2-point calibration and a master curve provided via the reagent barcode. The results obtained were then expressed as µg/dl.

2.5.2 Estimation of Total carbohydrate

Total carbohydrate content was estimated by the method as prescribed [12]. The 0.5% homogenate of liver and 10% homogenate of gills, kidney and muscle tissues were prepared in 10% TCA and centrifuged at 1000 × g for 15 minutes. To 0.2 ml supernatant, 5 ml of anthrone reagent was added and boiled for 15 minutes. The tubes were cooled and the absorbance was read at 620 nm in spectrophotometer against a reagent blank. The standards were also treated similarly. The values were expressed as mg of glucose / g wet wt. of tissue.

2.5.3 Assay of Glucose 6-phosphatase (EC 3.1.3.9)

Glucose 6-phosphatase was assayed according to the method prescribed [13]. The 10 % homogenate of liver tissue was prepared in 0.33 M sucrose solution and centrifuged at 11,000×g for 30 minutes in a refrigerated centrifuge. The supernatant obtained was again centrifuged for 60 minutes at 10,500×g and the supernatant was discarded. The pellet was suspended in ice-cold 0.33 M sucrose solution and homogenized in a glass-Teflon homogenizer. The homogenate obtained was used as the enzyme source. The incubation mixture in a total volume of 1ml contained 0.3 ml of buffer, 0.5 ml of substrate and 0.2 ml of enzyme preparation. The incubation was carried out at 37°C for 60 minutes. Arrested the reaction by the addition of 1 ml of 10% TCA and centrifuged. The phosphorus content of the supernatant was estimated by the method as prescribed [13].The enzyme activity was expressed as µg of inorganic phosphorus liberated / min / mg protein.

2.5.4 Estimation of Blood Glucose

Blood Glucose was estimated by the method as prescribed [14]. To 0.2 ml of blood, 0.8 ml of 10 % TCA was added. The contents were mixed well. The tubes were centrifuged at 1000×g for 5 minutes. 0.5 ml of supernatant was taken. To this 2.0 ml of ortho- toluidine reagent was added. The tubes were then heated in a boiling water bath for 15 minutes. The standards were also treated in the same manner along with the reagent blank. The values were expressed as mg glucose / dl.

2.5.5 Assay of Lactate Dehydrogenase (LDH) (EC 1.1.1.27)

Lactate Dehydrogenase was assayed according to the method as prescribed [15]. To 1.0 ml of the buffered substrate, added 0.2 ml of sample and incubated at 37° C for 15 minutes. After adding 0.2 ml of NAD^+ solution, continued the incubation for another 30 minutes and then added 1.0 ml of DNPH reagent. Incubated the mixture for a period of 15 minutes at 37°C. Then added 7.0 ml of 0.4 N NaOH solution and measured the colour developed at 520 nm in a spectrophotometer. Treated the standards also in the same manner along with blank. The enzyme activity was expressed as µ moles of pyruvate liberated / h / mg protein.

2.5.6 Estimation of Pyruvate

Pyruvate was estimated by the method as prescribed [16]. About 5% homogenate of liver and 10% homogenate of gills, kidney and muscle tissues were prepared in 10% TCA and centrifuged at 1000× g for 15 minutes. To 2.0 ml of supernatant, 0.5 ml of 0.1% 2, 4-DNPH reagent was added and the tubes were kept at room temperature for 5 minutes and 3.0 ml of 2.5 N NaOH solution was added. After 10 minutes the absorbance was read at 540 nm in a spectrophotometer against a reagent blank. The blank consisted of 2.0 ml of 10% TCA, 0.5 ml of 0.1% 2, 4-DNPH and 3.0 ml of 2.5 N NaOH solutions. Treated the standards also in the same manner. The values were expressed as µ moles of pyruvate / g wet wt. of tissue.

2.5.7 Assay of Alanine aminotransferase (ALT) (EC 2.6.1.2)

Alanine aminotransferase was assayed by the method as prescribed [17]. About 10% homogenate of gills, liver, heart, kidney and muscle were prepared in 0.33 M sucrose solution and centrifuged at 1000× g for 15 minutes. The supernatant obtained was used as the enzyme source. Pipetted out 1ml buffered substrate into 'test' and 'control'. Added 0.2 ml of the enzyme source into the 'test' and incubated the tubes at 37 ° C for 60 minutes. After incubation, 0.2 ml enzyme was added to the control. 1ml of 2, 4 - DNPH reagent was added and kept at room temperature for 20 minutes. The reaction was stopped by the addition of 10 ml of 0.4 N NaOH, vortexed and kept at room temperature for 5 minutes. The absorbance was measured at 540 nm in a spectrophotometer against a reagent blank. The ALT activities were expressed as µ moles of pyruvate liberated / h / mg protein.

2.5.8 Assay of Aspartate aminotransferase (AST) (EC 2.6.1.1)

Aspartate aminotransferase was assayed by the method as prescribed [18]. About 10% homogenate of gills, liver, heart, kidney and muscle were prepared in 0.33 M sucrose solution and centrifuged at 1000× g for 15 minutes. The supernatant obtained was used as the enzyme source. Pipetted out 1ml buffered substrate into 'test' and 'control'. Added 0.2 ml of the enzyme source into the 'test' and incubated the tubes at 37 °C for 60 minutes. After incubation, 0.2 ml enzyme was added to the control. 1ml of 2, 4 - DNPH reagent was added and kept at room temperature for 20 minutes. The reaction was stopped by the addition of 10 ml of 0.4 N NaOH, vortexed and kept at room temperature for 5 minutes. The absorbance was measured at 540 nm in a spectrophotometer against a reagent blank. The AST activities were expressed as µ moles of pyruvate liberated / h / mg protein.

2.5.9 Assay of Alkaline phosphatase (ALP) (EC 3.1.3.1)

Alkaline phosphatase was assayed by the method as prescribed [19]. About 10% homogenate of gills, liver,

heart, kidney and muscle were prepared in 0.33 M sucrose solution and centrifuged at 1000×g for 15 minutes. The supernatant obtained was used as the enzyme source. Pipetted out 1ml buffered substrate into 'test' and 'control'.and incubated for a few minutes at 37 °C. Then added 50µl of enzyme source to the 'test'. Again incubated at 37°C for 15 minutes. Added 0.8 ml of NaOH and 1.2 ml of $NaHCO_3$ to both the tubes. Then added 50µl of enzyme source to the 'control'. This was followed by the addition of 1 ml of 4 - aminoantipyrine and 1 ml of potassium ferricyanide to both the tubes. Read the absorbance at 520 nm. Treat the blank and standards also similarly. The values were expressed as mg of phenol liberated /min / mg protein.

2.5.10 Assay of serum Acid Phosphatase (ACP) (EC 3.1.3.2)

Acid Phosphatase was assayed by the method as prescribed [13]. Mixed 1.5 ml of citrate buffer, 1.0 ml of substrate and 0.2 ml of serum together. Incubated the reaction mixture at 37°C for 15 minutes. Terminated the reaction by the addition of 1.0 ml of Folin & Ciocalteu's reagent. Incubated the controls without enzyme source and added the enzyme source after the addition of Folin -Ciocalteu Phenol reagent. Then added 1.0 ml of 15% sodium carbonate solution and incubated for a further 10 minutes at 37 ° C. Read the blue colour developed at 640 nm against a blank. A set of graded volumes of phenol standards were also run simultanously. The activity of the enzyme was expressed as mg of phenol liberated / min / mg protein.

2.5.11 Assay of Glutamate dehydrogenase (GDH) (E.C.1.4.1.3)

Glutamate dehydrogenase was assayed by the method as prescribed [20]. About 10% homogenate of gills and 5% homogenate of liver, kidney and muscle were prepared in 0.33 M sucrose solution and centrifuged at 1000×g for 15 minutes. The supernatant obtained was used as the enzyme source. The reaction mixture consisted of 2.1 ml phosphate buffer, 0.2 ml enzyme source, 0.1 ml NADH, 0.2 ml Ammonium acetate, 0.2 ml EDTA and 0.1 ml Triton X-100. The above mixture was equilibrated at room temperature for 10 minutes. Started the reaction by adding 0.1 ml of 2-oxoglutarate and the rate of change of extinction at 340 nm with time were noted. Molar Extinction Coefficient of NADH is 6.3×10^3 litres mol^{-1} cm^{-1}. The enzyme activity was calculated as micromoles of NADH oxidized / minute / mg protein.

2.5.12 Estimation of Protein

Protein was estimated by the method as prescribed [21]. Pipetted out 0.2 ml of tissue homogenate to the test tube and added 1 ml of 10 % TCA. The tubes were centrifuged at 5000 × g for 10 minutes. The supernatant was discarded and the precipitate was dissolved in 1 ml of 0.1 N NaOH. Added 5 ml of alkaline copper reagent and kept for 10 minutes at room temperature. After 10 minutes added 0.5 ml Folin- Ciocalteu Phenol Reagent and kept in dark for 30 minutes. The absorbance was read at 620 nm against a reagent blank. A set of graded volumes of protein standard were also run simultaneously. The values are expressed as mg of protein/ g wet wt. of tissue.

3. RESULTS

In the present investigation, it was found that different biochemical parameters and enzymes associated with metabolic pathways 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 *Clarias batrachus*. The results of each of the biomarker assessed are as follows:

3.1 Lethal Toxicity Study

The calculated LC_{50} value for phenol and m-cresol exposure in *C. batrachus* over periods of up to 96 hours was found to be 28.45 mg l^{-1} and 25.7 mg l^{-1} respectively. The results are presented in **Table 3.1**.

Phenolic Compound (s)	Acute Toxicity Range (mg/l)		Median LC50 (mg/l)
Phenol	32.50	24.40	28.45
m-cresol	26.76	24.64	25.7

Table 1: LC50 value for phenol and m-cresol in *Clarias batrachus* (up to 96 h)

3.2 Serum Cortisol

Among phenol treated group showed the least cortisol level. The results are shown in **Table 3.2.**

Cortisol level	Control (µg / dl)	Phenol (µg / dl)	m-cresol (µg / dl)
	25.60	16.76	18.56

Table 3.2 Effect of different phenolic compounds on cortisol in *Clarias batrachus.* Values in the same row with different upper case letters vary significantly (P<0.05) between treatment groups.

3.3 Total Carbohydrates

A statistically significant decrease in total carbohydrate (P<0.05) was observed in liver and muscle of both the treated groups compared to control. Among the tissues kidney showed a statistically significant elevated carbohydrate level (P<0.05) in both the treated groups compared to control. No significant variation was observed in gills of both the treated groups compared to control. The results of effect in carbohydrate concentration is shown in

Tissue	**Total Carbohydrate (mg/g wet weight of tissue)**		
	Groups treated with phenolic compounds		
	Control	**Phenol**	**m-cresol**
Gills	4.46 ± 0.49	4.34 ± 0.01	4.48 ± 0.02
Liver	45.66 ± 2.23	40.30 ± 1.78	30.22 ± 1.76
Kidney	3.11 ± 0.70	3.62 ± 0.81	3.81 ± 0.64
Muscles	17.45 ± 2.39	11.04 ± 1.91	13.4 ± 0.96

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 (Two-way ANOVA).
*Each value represents the mean ± S.D of three separate experiments

Table 3.3: Effect of different phenolic compounds on total carbohydrate concentration in different body tissues of *Clarias batrachus*.

3.4 Glucose 6 phosphatase

There was a significant decrease (P<0.05) in glucose-6-phosphatase activity in both the treated groups compared to control. The results are shown in **Table 3.4**.

Groups	Control	Phenol	m-cresol
Glucose 6 phosphatase activity	8.50± 1.11	4.56± 0.60	3.33± 0.77

*Each value represents the mean ± S.D of three separate experiments.

Table 3.4: Effect of different phenolic compounds on glucose-6-phosphatase activity (mean ± S.D) in *Clarias batrachus*.

3.5 Blood Glucose

There was a prominent decrease in blood glucose concentration after exposure to phenolic compounds. The results are shown in **Table 3.5**

Groups	Control	Phenol	m-cresol
Blood glucose	50.25± 1.11	40.0± 0.60	35.35± 0.77

*Values are expressed as mg/dl; Each value represents the mean ± S.D of three separate experiments.

Table 3.5: Effect of different phenolic compounds on blood glucose (mean ± S.D) in *Clarias batrachus*.

3.6 Serum pyruvate

Two-factor ANOVA followed by Tukey's test showed that there was significant variation (P<0.05) in pyruvate level between treatments and also between tissues **(Table 3.6).** Gills, liver and kidney of both the treated groups showed a significantly increased pyruvate level (P<0.05) compared to control. In both the treated groups muscle showed a significantly decreased pyruvate level (P<0.05) compared to control.

Tissue	Pyruvate (micro moles /g wet weight of tissue)		
	Groups treated with phenolic compounds		
	Control	Phenol	m-cresol
Gills	3.85 ± 0.49	4.24 ± 0.01	4.48 ± 0.02
Liver	7.36± 2.23	10.24 ± 1.78	10.56 ± 1.76
Kidney	5.14 ± 0.70	7.62 ± 0.81	7.04 ± 0.64
Muscles	5.16 ± 2.39	9.43 ± 1.91	9.56. ± 0.96

Values are expressed as µmoles of pyruvate / g wet wt of tissue. Each value represents the mean ± S.D of three separate experiments.

Table 3.6: Effect of different phenolic compounds on level of pyruvate (mean ± S.D) in *Clarias batrachus.* 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.

3.7 Lactate Dehydrogenase

LDH activity in different tissues of *Clarias batrachus* treated with different phenolic compounds showed significant variations (P<0.05) compared to control **(Table 3.7).** In the phenol treated group, tissues such as liver, kidney and muscle showed significantly elevated activity (P<0.05) compared to control. Among the tissues of m-cresol treated group the gills and muscle showed a significantly elevated activity (P<0.05) and the liver and kidney showed a significantly decreased activity (P<0.05) compared to control.

Tissue	LDH activity		
	Groups treated with phenolic compounds		
	Control	Phenol	m-cresol
Gills	2.16 ± 0.49	1.68 ± 0.01	1.85 ± 0.02
Liver	2.67± 2.23	4.34 ± 1.78	2.25 ± 1.76
Kidney	3.14 ± 0.70	3.78 ± 0.81	2.56 ± 0.64
Muscles	7.09 ± 2.39	9.43 ± 1.91	10.56 ± 0.96

*Values are expressed as µmoles of LDH liberated / h / mg protein; Each value represents the mean ± S.D of three separate experiments.

Table 3.7: Effect of different phenolic compounds on LDH activity (mean ± S.D) in *Clarias batrachus.* 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.

3.8 ALT/SGPT

Two-factor ANOVA followed by Tukey's test showed that there was significant elevation in ALT activity (P<0.05), in both the treated groups compared to control **(Table 3.8).** Liver and kidney of phenol treated group showed significantly elevated activity (P<0.05) compared to m-cresol treated group. Gills and muscle of m-cresol treated group showed significantly elevated activity compared to phenol treated group.

Tissue	ALT/SGPT activity		
	Groups treated with phenolic compounds		
	Control	Phenol	m-cresol
Gills	1.16 ± 0.49	1.26 ± 0.01	1.10 ± 0.02
Liver	3.68± 2.23	10.12 ± 1.78	7.56 ± 1.76
Kidney	2.80 ± 0.70	8.23 ± 0.81	7.23 ± 0.64
Muscles	2.50 ± 2.39	3.09 ± 1.91	3.90 ± 0.96

*Values are expressed as µmoles of ALT liberated / h / mg protein; Each value represents the mean ± S.D of three separate experiments.

Table 3.8: Effect of different phenolic compounds on ALT/SGPT activity (mean ± S.D) in *Clarias batrachus.* 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.

3.9 AST/SGOT

AST activity was found to be significantly elevated (P<0.05) in tissues such as liver, kidney and muscle of both the treated groups compared to control. In the phenol treated group liver and kidney showed significantly elevated AST activity (P<0.05) compared to m-cresol treated group. Gills of both the treated groups did not show any significant variation compared to control **(Table 3.9)**.

Tissue	AST/SGOT activity		
	Groups treated with phenolic compounds		
	Control	Phenol	m-cresol
Gills	1.26± 0.49	1.31± 0.01	1.14± 0.02
Liver	3.68± 2.23	10.22± 1.78	7.56± 1.76
Kidney	2.75± 0.87	8.56± 0.81	7.28± 0.64
Muscles	2.50 ± 2.39	3.09± 1.91	3.93± 0.68

*Values are expressed as µmoles of AST liberated / h / mg protein; Each value represents the mean ± S.D of three separate experiments.

Table 3.9: Effect of different phenolic compounds on AST/SGOT activity (mean ± S.D) in *Clarias batrachus.* 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.

3.10 ALP/Alkaline Phosphatase

Statistical analysis showed significant variations in ALP activity (P<0.05) in all the treated groups compared to control **(Table 3.10).** Gills, kidney and muscle of both the treated groups showed significantly elevated ALP activity (P<0.05) compared to control. Liver of m-cresol treated group showed a significantly elevated activity (P<0.05) whereas the liver of phenol treated group did not show any significant variation compared to control.

Tissue	ALP activity		
	Groups treated with phenolic compounds		
	Control	Phenol	m-cresol
Gills	3.18 ± 0.49	4.08 ± 0.01	8.60± 0.02
Liver	7.42 ± 2.23	7.58± 1.78	10.42± 1.76
Kidney	4.98 ± 0.87	6.38 ± 0.81	7.13± 0.64
Muscles	2.72± 2.39	6.20± 1.91	3.93± 0.68

*Values are expressed as µmoles of ALP liberated / h / mg protein; Each value represents the mean ± S.D of three separate experiments

Table 3.10: Effect of different phenolic compounds on ALP activity (mean ± S.D) in *Clarias batrachus.* 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.

3.11 Acid Phosphatase

One-way ANOVA followed by Turkey's test showed that there was significant increase in serum acid phosphates activity (P<0.05) in both the treated groups compared to control **(Table 3.11).**

Groups	Control	Phenol	m-cresol
Serum acid phosphatase	20± 1.11	60.0± 0.60	40.25± 0.77

*Values are expressed as U/L

Table 3.11: Effect of different phenolic compounds on serum acid phosphatase (mean ± S.D) in *Clarias batrachus*.

3.12 Glutamate Dehydrogenase

GDH activity was found to show statistically significant variations (P<0.05) in all the treated groups compared to control **(Table 3.12).** In the phenol treated group, tissues such as gills, liver and kidney showed a statistically significant elevated activity (P<0.05) compared to control. Both gills and liver of the m-cresol treated group showed a statistically significant elevated activity (P<0.05) compared to control. Muscle of both the treated groups showed a statistically significant decreased activity (P<0.05) compared to control.

Tissue	Glutamate dehydrogenase activity		
	Groups treated with phenolic compounds		
	Control	Phenol	m-cresol
Gills	0.053 ± 0.49	0.092 ± 0.01	0.062± 0.02
Liver	0.086 ± 2.23	0.126± 1.78	0.097± 1.76
Kidney	0.062 ± 0.87	0.079 ± 0.81	0.052± 0.64
Muscles	0.052± 2.39	0.033± 1.91	0.024± 0.68

Values are expressed as µmoles of IU/mg protein. Each value represents the mean ± S.D of three separate experiments

Table 3.12: Effect of different phenolic compounds on Glutamate dehydrogenase activity (mean ± S.D) in *Clarias batrachus.* 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.

3.13 Total Serum Protein

Statistically significant decreased protein level (P<0.05) was observed in liver and muscle of both the treated groups compared to control **(Table 3.13).** Gills and kidney of both the treated groups showed a significantly elevated protein level (P<0.05) compared to control.

Tissue	Total serum proteins		
	Groups treated with phenolic compounds		
	Control	Phenol	m-cresol
Gills	42.76 ± 0.49	45.32 ± 0.01	47.61± 0.02
Liver	70.00 ± 2.23	58.56± 1.78	56.67± 1.76
Kidney	32.53 ± 0.87	35.28 ± 0.81	38.67± 0.64
Muscles	51.06± 2.39	38.09± 1.91	35.12± 0.68

*Values are expressed as mg protein/g wet weight of tissue; Each value represents the mean ± S.D of three separate experiments.

Table 3.13: Effect of different phenolic compounds on total proteins (mean ± S.D) in *Clarias batrachus.* 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.

4. DISCUSSION

In the present study, decreased cortisol level was observed in both phenol and m-cresol treated fishes compared to control. There are several studies which provided evidence that the capacity to raise plasma cortisol is impaired in fish exposed to organic pollutants [22] and metals [23-25]. As cortisol is involved in the regulation of physiological functions that helps the animal to cope with stress, inhibition of the pituitary-interrenal axis will impair the ability of the animal to cope with stressors [26]. Prolonged exposure to pollutants may lead to hyperactivity, and as a result in the exhaustion of the pituitary-interrenal axis [27]. The lack of cortisol response suggests that, similar to other xenobiotics phenol and m-cresol can act as an endocrine disruptor and as such impair steroidogenesis. It is not known how phenolics can affect cortisol production. However there are some possible explanations. First, it might be possible that one of the primary steps in the steroid hormone synthesis pathway was compromised. Total carbohydrate content was found to be decreased in liver and muscle of both the treated groups compared to control. Chemical stress causes rapid depletion of stored carbohydrates primarily in liver and other tissues [28]. Inhibition of glucose-6-phosphatase activity was found in the liver of both the treated groups compared to control. Inhibition of glucose-6-phosphatase activity may be a reflection of damage to the microsomal membrane as the enzyme is localized exclusively in the membranes of the endoplasmic reticulum. On exposure to phenolic compounds gills, liver and kidney showed an elevated pyruvate level compared to control. This might be due to the higher glycolysis rate, which is the only energy-producing pathway for the animal when it is under stress conditions. The end product of the glycolytic pathway is pyruvate. Pyruvate

occupies an important junction between various metabolic pathways. It may be decarboxylated to acetyl CoA which can enter the TCA cycle or it may be utilized for fatty acid synthesis. Pyruvate may be carboxylated to oxaloacetate which can be used for gluconeogenesis. Muscle of both the treated groups showed a decreased pyruvate level compared to control.

Lactate dehydrogenase is an enzyme recognized as a potential marker for assessing the toxicity of a chemical. LDH is an anaerobic enzyme involved in the conversion of pyruvate to lactate in glycolysis. The LDH in the liver and kidney of fishes treated with phenol showed an elevated activity compared to control. The studies reported a similar increase in LDH activity in juvenile Australian Bass and *Macquaria novemaculeata* in response to two different crude oil spills [29]. The increase in LDH activity also suggests a significant increase in the conversion of pyruvate to lactic acid, thereby leading to the accumulation of lactic acid. Compared to control a significant decrease in LDH activity in liver and kidney of m-cresol treated fishes and in gills of fishes treated with phenol was observed. This may be due to increased tissue damage. Similar results were obtained when *Clarias batrachus* were exposed to sub-lethal concentrations of organophosphorus insecticide [30]. Several reports revealed decreased LDH activity in tissues under various toxic conditions [31, 32]. LDH is an important glycolytic enzyme in biological systems and is inducible by oxygen stress. Therefore, the activity of several regulatory enzymes may be altered in order to meet the required energy demands under toxic stress [8] including the activity of lactate dehydrogenase (LDH), which sustains the continued process of glycolysis under anaerobic conditions [33]. Several reports revealed decreased LDH activity in tissues under various toxic conditions [31, 32]. ALT is an enzyme frequently used in the diagnosis of damage caused by pollutants in various tissues such as liver, muscle, and gills [34, 35]. This enzyme is known to play a key role in mobilizing L-amino acids for gluconeogenesis and function as links between carbohydrate and protein metabolism under altered physiological, pathological and induced environmental conditions. Elevation in the levels of AST and ALT in different tissues of *C. batrachus* can be considered as a response to the stress induced by phenolic compounds to generate keto acids like α-ketoglutarate and oxaloacetate for contributing to gluconeogenesis and/or energy production necessary to meet the excess energy demand. Alkaline phosphatase and acid phosphatase catalyses the hydrolysis of monophosphate esters and has a wide substrate specificity. The activity of ALP has been significantly elevated in all the tissues (gills, liver, kidney and muscle) treated with phenol and m-cresol compared to control. Increased ALP activity may be due to pathological processes such as liver impairment, kidney dysfunction and bone disease [36]. Phosphatases play major roles in the moulting physiology of many fishes. Serum acid phosphatase showed an elevated activity compared to control. An elevation in ACP activity suggests an increase in lysosomal mobilization and cell necrosis due to the toxicity of phenolics. This increase also suggests the supply of phosphate group for energy metabolism. This shows an adverse impact on metabolism, which may lead to negative impact on growth, health and reproduction. Degeneration and necrosis induced in hepatic parenchymatous cells by these toxicants may cause release of acid phosphatase in the serum. Alterations in ALP and ACP activities in tissues and serum have been reported in pesticide treated fish [37]. Increase in the levels of ALP and AST has been shown to reflect liver damage, whereas an elevation in the ALP activity may be indicative of renal and liver damage [38, 39].

GDH activity was found to be elevated in almost all tissues treated with phenol compared to control. This increased activity may have helped in funneling more α-ketoglutarate into TCA cycle for more energy generation. Whereas in fishes treated with m-cresol tissues such as liver and gills showed almost constant activity but kidney and muscle showed a decreased activity compared to control. An inhibition of GDH activity in gills, brain, kidney and liver of fishes exposed to toxicants was observed [40]. Fishes exposed to sub-lethal concentrations of different phenolic compounds showed alterations in protein content in different tissues compared to control. Gills and kidneys of both the treated group showed increased protein content compared to control. Liver and muscle of both the treated groups showed decreased protein content compared to control. The reduction in protein content indicates that under stress conditions the tissue protein may undergo proteolysis, which may have resulted in the production of free amino acids which can be used in the tricarboxylic acid cycle for energy production. This would lead to an increased free amino acid pool [41] which can be used for ATP production by transamination reactions or by gluconeogenic pathway.

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Conflict of Interest: None Declared

Cite this article as:
Amit Alexander, OP Verma, Abhishek Mathur, Abhishek Sharan, PK Varshney. Evaluation of Changes in Metabolic parameters and Enzymes Involved in Metabolic Pathways in *Clarias batrachus* after Exposure to Phenolic Compounds. Asian Journal of Biomedical and Pharmaceutical Sciences, 2013, 3: (21), 60-67.

