Evaluation of Antioxidant profile of *Labeo rohita* in stress condition after exposure to phenolic compounds

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Abstract: Phenolic compounds may be the part of oils, hydrocarbons and different types of insecticides, herbicides, pesticides and industrial effluents which are released in the fresh water bodies knowingly harming the aquatic life in terms of flora and fauna. Relatively little is known about the effects of low concentrations of phenolic compounds on the normal physiological functions of freshwater fishes. Phenolic compounds present in sub-lethal concentrations in water might enter into the blood stream of these fishes through the gills or the mucus epithelium of the mouth and finally be distributed in different organs of the body which in turn creates the stress-like conditions in these fishes, thus leads to disturbances in the antioxidant enzymes concentration and levels within their body. The present study was performed artificially in a separate pond having the healthy fishes numbers of Labeo rohita (Rohu), a common edible fish. The 50 % lethal concentration (LC_{50}) of the doses were determined for both o-cresol and m-cresol Then after, 2440 mg/ml of phenol and 2464 mg/ml of m-cresol were dosed separately in L. rohita fishes groups. Fishes were transferred to tanks containing no toxicants were utilized 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 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 while on exposure to m-cresol, the results of increased activity of SOD enzyme was: liver>gills>kidney>muscles. Changes in the levels of superoxide dismutase have been detected in fishes exposed to various degrees of oxygen tension. 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. 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. 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. 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. The study suggested that, under stress conditions such as on exposure to phenolic compounds or death causing chemicals, fishes such as Labeo rohita have elevated and variable activity in different body tissues of antioxidant markers which makes them adaptive to fight against stressful conditions.

Keywords: Stress, phenolic compounds, antioxidant profile, markers, L. rohita.

1. INTRODUCTION

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 [1-3]. The aquatic environment is particularly sensitive to the toxic effects of contaminants since a considerable amount of the chemicals used in industry, urbanization, and in agriculture enter marine and other aquatic environments. The stressors in the environment exert their adverse effects at the organismal level leading to impaired physiological functions in aquatic organisms. Xenobiotics are potentially harmful to fish by inducing tissue damage in gill, kidney and liver [4] growth retardation [5], genotoxicity [6], reproductive disturbances [7], tissue bioaccumulation [8-9]. Since the second half of the last century, the environment has been contaminated by numerous xenobiotics; amongst these phenolics are of special concern. Phenolic compounds are environmentally important due to their extensive use in various industries, presence in wastewaters and their potential toxicity. These lipophilic compounds have numerous industrial applications, which enhance the risk to the environment and to human health [10]. . Phenolic compounds are used in the manufacture of many agricultural pesticides [11]. They can also be

introduced into the environment through degradation of natural substances [12] and industrial activities (e.g., dyes, plastics, pharmaceuticals and explosives) [13, 14]. Phenolic compounds can cause toxicity, with bioaccumulation effects in animals and plants [15]. The present study is describing the changes in antioxidant markers in *Labeo rohita* after exposure to phenolic compounds.

II. MATERIALS AND METHODS

Chemicals and Reagents used for the study

The chemicals and reagents used for the study were of analytical grade and The diagnostic kits used in the study for biochemical testing were from standard diagnostics company.

Experimental design

(i) Collection and maintenance of test fish

Labeo rohita (20-25g) were collected from the culture ponds of Yamuna river of Delhi region, India and were brought to artificial small ponds/aquarium in Institute of Transgene Life Sciences, Dehradun/Lucknow units. Further, these fishes were kept in large tanks where a continuous and gentle flow of fresh water was maintained. 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} determinations were carried out by following semi-static acute toxicity test. For the experiment, 6 fishes were transferred to large experimental tubs, each containing 18 liters of dechlorinated tap water. The 50 % lethal concentrations (LC50) of the doses were determined for both o-cresol and m-cresol Fishes transferred to tanks containing no toxicants were utilized 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.

Preparation of tissue samples for the study

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

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

Superoxide dismutase in different tissues was determined using the method [16].

Assay of Catalase (CAT) (E.C.1.11.1.6)

Catalase level in different tissues was determined using the method [17].

Assay of Glutathione peroxidase (GPx) (E.C. 1.11.1.9)

Glutathione peroxidase in different tissues was estimated by the method [18].

Assay of Glutathione-S-transferase (GST) (E.C.2.5.1.18)

Glutathione-S-transferase in different tissue were determined using the method [19].

Estimation of total reduced glutathione (GSH)

Total reduced glutathione were estimated by the method [20].

Estimation of Conjugated dienes (CD)

The concentration of conjugated dienes was estimated according to the method [21].

Estimation of Hydro-peroxides (HP)

Hydro peroxide was estimated by method [22].

III. RESULTS AND DISCUSSION

It was observed that, the malfunctioning of different organs in *Labeo rohita* occurs due to the alterations in different parameters and enzymes. The results thus show the abnormal and lethargic effect of phenolic compounds as pollutants on *Labeo rohita.* .

Acute toxicity range

The acute toxicity doses of phenol and m-cresol were found to be 3212 and 2957 mg/l respectively. The results are shown in **Table 1** and **Figure 1**.

Superoxide dismutase (SOD)

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 *Labeo rohita* 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. The results are shown in **Table 2** and **Figure 2**.

Catalase (CAT)

In the present study catalase activity in different tissues of *L. rohita* treated with different phenolic compounds showed significant variations ($P <0.05$), compared to control group. Tukey'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. The results are shown in **Table 3** and **Figure 3**.

Glutathione peroxidase (GPx)

Glutathione peroxidase activity showed an overall significant change ($P<0.05$) in experimental groups of animal 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. The results are shown in **Table 4** and **Figure 4**.

Glutathione S- transferase (GST)

In the present study, Glutathione-S-transferase activity in different tissues of *Labeo rohita* treated with different phenolic compounds showed significant variations ($P<0.05$), compared to control group. 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. The results are shown in **Table 5** and **Figure 5**.

Total reduced glutathione (GSH)

Two-factor ANOVA followed by Turkey's test showed that there was significant ($P<0.05$), 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. The results are shown in **Table 6** and **Figure 6**.

Conjugated dienes (CD)

Conjugated diene level in all the phenolic compounds treated groups was significantly ($P<0.05$) 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. The results are shown in **Table 7** and **Figure 7**.

Hydrogen peroxides (HP)

The level of hydro peroxides in the groups treated with both the phenol and m- cresol showed statistically significant (P<0.05) 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 hydro peroxide was seen in liver. The results are shown in **Table 8** and **Figure 8**.

IV. CONCLUSION

In the present study, almost all the tissues treated with phenol and m-cresol for 21 days in *Labeo rohita* showed significantly elevated SOD and CAT activity compared to control. 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. For the detoxification of increased H2O2 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. 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. Increased GST activity indicates the role of this enzyme in protection against the toxicity of xenobiotic-induced lipid per oxidation. Increased GST activity indicates the role of this enzyme in protection against the toxicity of xenobiotic-induced lipid per oxidation. Many studies analyzing GST in liver of fish exposed to different insecticides showed an enzymatic induction. 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. The low GPx activity might be due to a direct phenol inhibition of enzyme synthesis or due to increased generation of hydro peroxide 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. Significantly elevated GPx activity in muscle shows that an induction in glutathione peroxidase activity has occurred in this tissue. 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. 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 per oxidative product and is an accurate indicator of lipid per oxidation and its elevated level indicated that lipid per oxidation has been initiated). An increased hydro peroxide 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 maybe because GPx catalyzes the reduction of H2O2 derived from oxidative metabolism as well as peroxides from oxidation of lipids and is considered the most effective enzyme against lipid per oxidation.

V. REFERENCES

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Table 1: Acute toxicity range of phenol and m-cresol in *L. rohita*

Phenolic Compound (s)	Acute Toxicity Range (mg/l)
Phenol	3212
m-cresol	2957

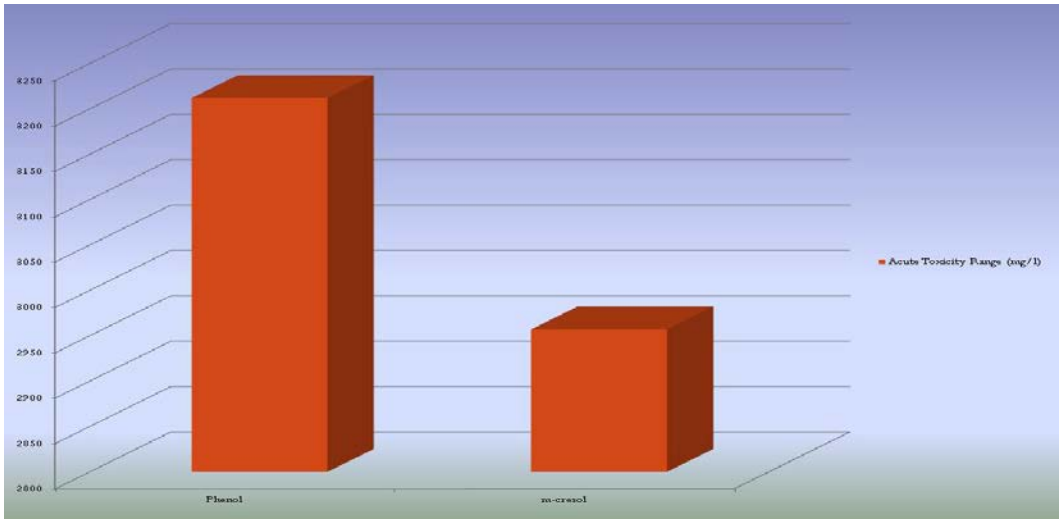

Figure 1: Acute toxicity range (LC50 values) of phenol and m-cresol in *L. rohita*

Table 2: Effect of phenolic compounds on SOD activity in different tissues of *L. rohita*

Tissue	SOD activity		
	Groups treated with phenolic compounds		
	Control	Phenol	m-cresol
Gills	12.76±2.73	23.32±2.76	05.61±2.83
Liver	12.70±1.10	27.56±1.10	15.67±1.10
Kidney	13.53±0.25	17.28±0.27	15.67±0.28
Muscles	3.06±0.15	02.09±0.27	04.12±0.28

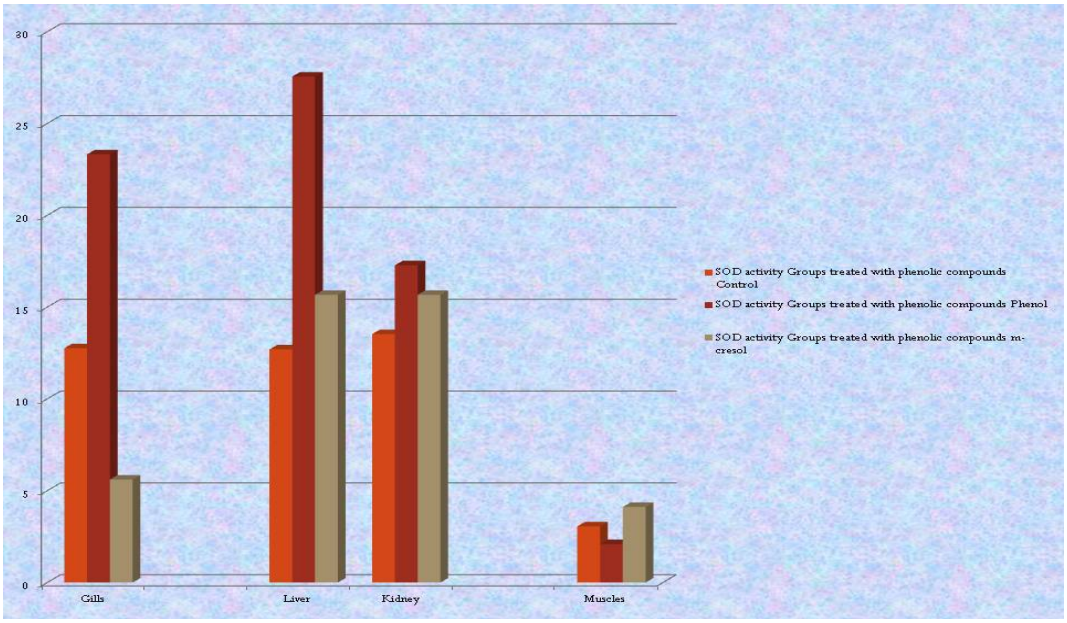

Figure 2: Effect of phenolic compounds on SOD activity in different tissues of *L. rohita*

Table 3: Effect of phenolic compounds on CAT activity in different tissues of L. rohita

Tissue	**CAT activity**		
	Groups treated with phenolic compounds		
	Control	**Phenol**	**m-cresol**
Gills	13.76±2.73	16.56±2.76	16.61±2.83
Liver	13.56±1.10	32.78±1.10	38.34±1.10
Kidney	4.93±0.25	8.28±0.27	11.23±0.28
Muscles	3.07±0.24	2.15±0.27	7.56±0.28

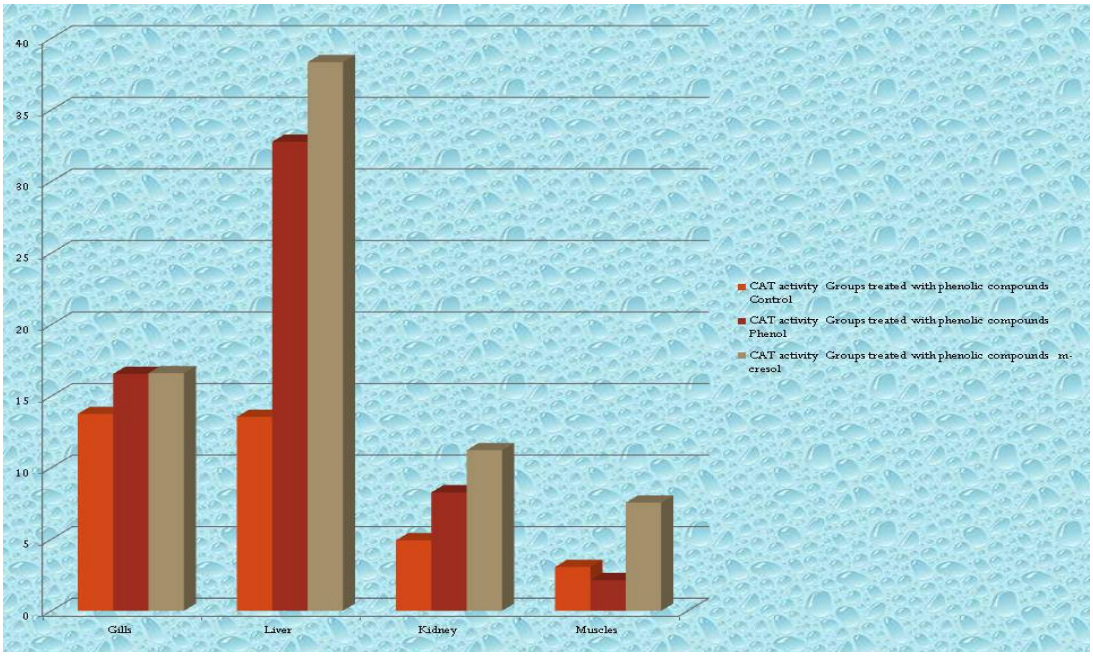

Figure 3: Effect of phenolic compounds on CAT activity in different tissues of *L. rohita*

Table 4: Effect of phenolic compounds on GPx activity in different tissues of *L. rohita*

Tissue	**GPx activity**		
	Groups treated with phenolic compounds		
	Control	**Phenol**	**m-cresol**
Gills	12.45±2.18	4.13±2.18	17.23±2.23
Liver	13.57±1.10	2.34±1.12	2.45±1.21
Kidney	11.21±0.27	5.56±0.57	10.25±0.26
Muscles	3.06±0.25	8.56±0.24	10.34±0.25

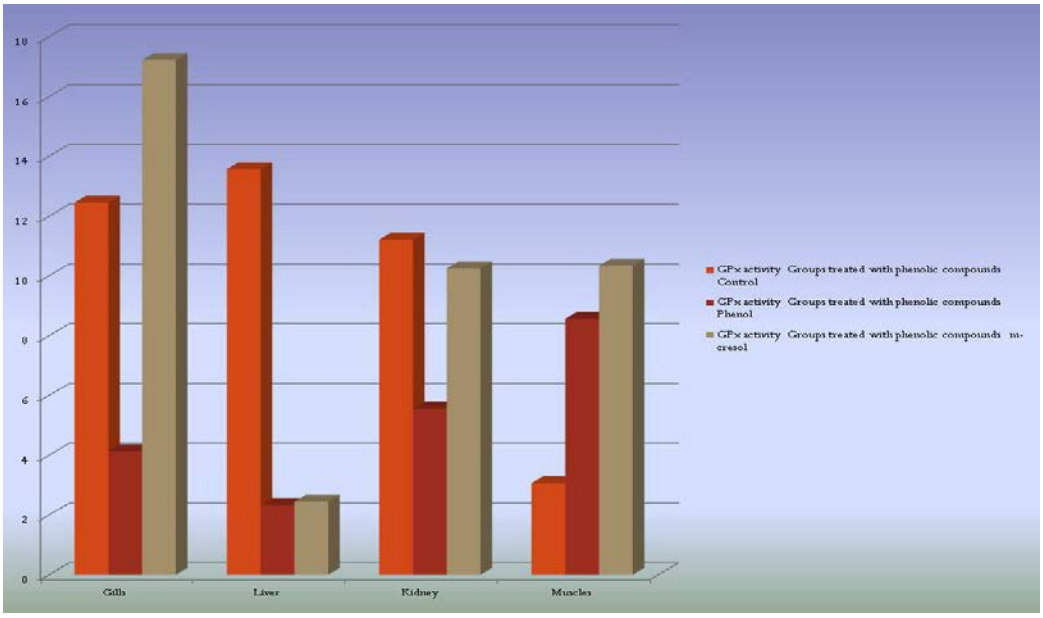

Figure 4: Effect of phenolic compounds on GPx activity in different tissues of *L. rohita*

Table 5: Effect of phenolic compounds on GST activity in different tissues of L. rohita

Tissue	**Glutathione S- Transferase activity**		
	Groups treated with phenolic compounds		
	Control	**Phenol**	**m-cresol**
Gills	23.23±2.56	36.65±2.45	20.21±2.07
Liver	35.34±1.18	47.23±1.21	61.78±1.23
Kidney	23.45±0.56	10.23±0.25	15.95±0.25
Muscles	7.34±0.35	5.57±0.29	3.23±0.22

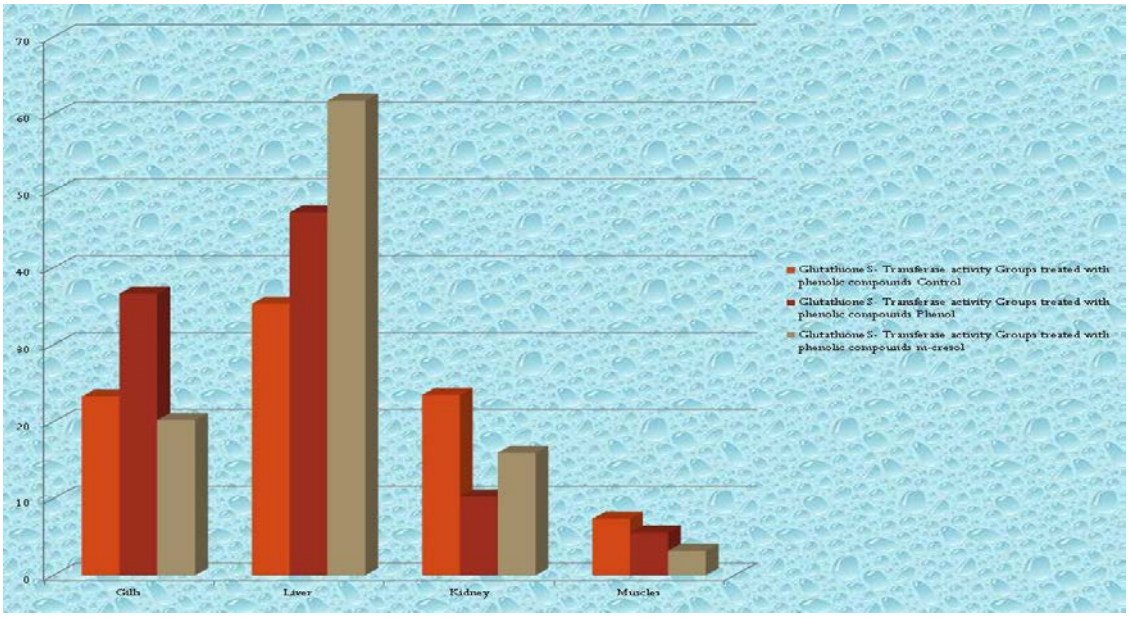

Figure 5: Effect of phenolic compounds on GST activity in different tissues of *L. rohita*

Table 6: Effect of phenolic compounds on GSH activity in different tissues of *L. rohita*

Tissue	**Total activity Reduced Glutathione (GSH)**		
	Groups treated with phenolic compounds		
	Control	**Phenol**	**m-cresol**
Gills	15.23±2.5	26.31±2.25	3.45±2.54
Liver	14.45±1.25	28.45±1.15	1.85±1.45
Kidney	11.23±0.34	9.56±0.26	7.75±0.26
Muscles	8.34±0.37	7.75±0.28	7.54±0.28

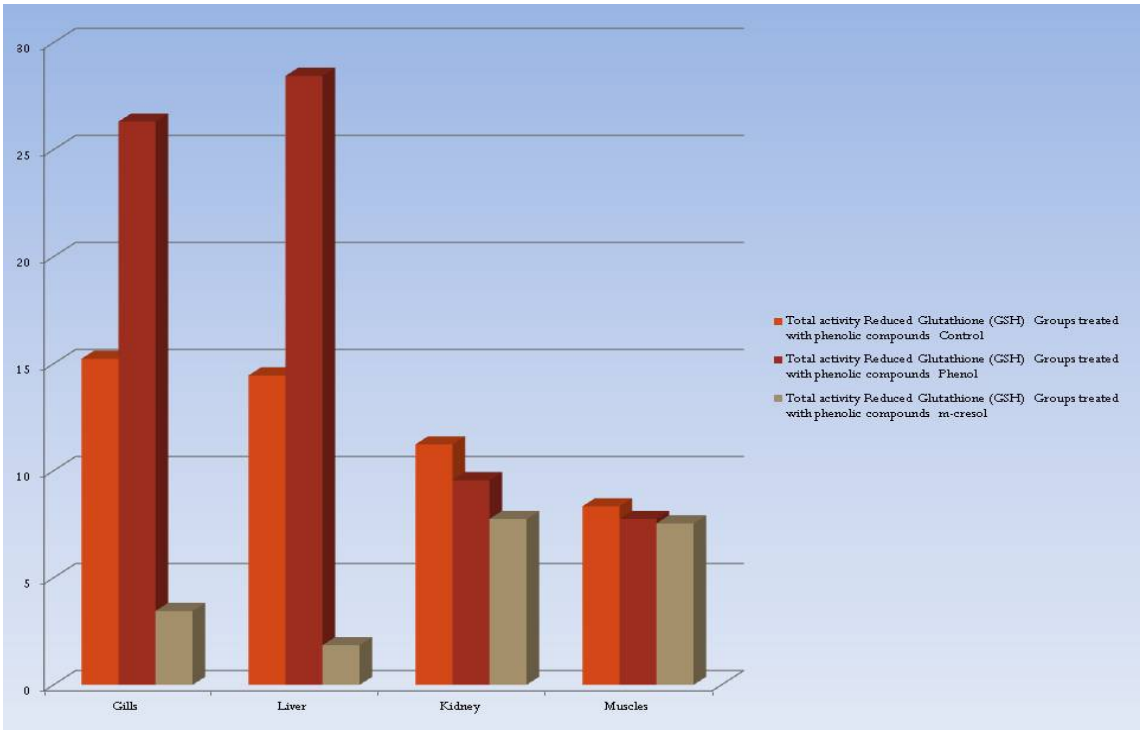

Figure 6: Effect of phenolic compounds on GSH activity in different tissues of *L. rohita*

Table 7: Effect of phenolic compounds on Conjugated dienes in different tissues of *L. rohita*

Tissue	**Conjugated dienes concentration**		
	Groups treated with phenolic compounds		
	Control	**Phenol**	**m-cresol**
Gills	37.67±2.67	47.13±2.34	41.23±2.85
Liver	38.56±1.15	50..32±1.15	52.21±1.15
Kidney	15.56±0.28	10.23±0.28	9.45±0.25
Muscles	23.43±0.25	21.32±0.25	27.34±0.25

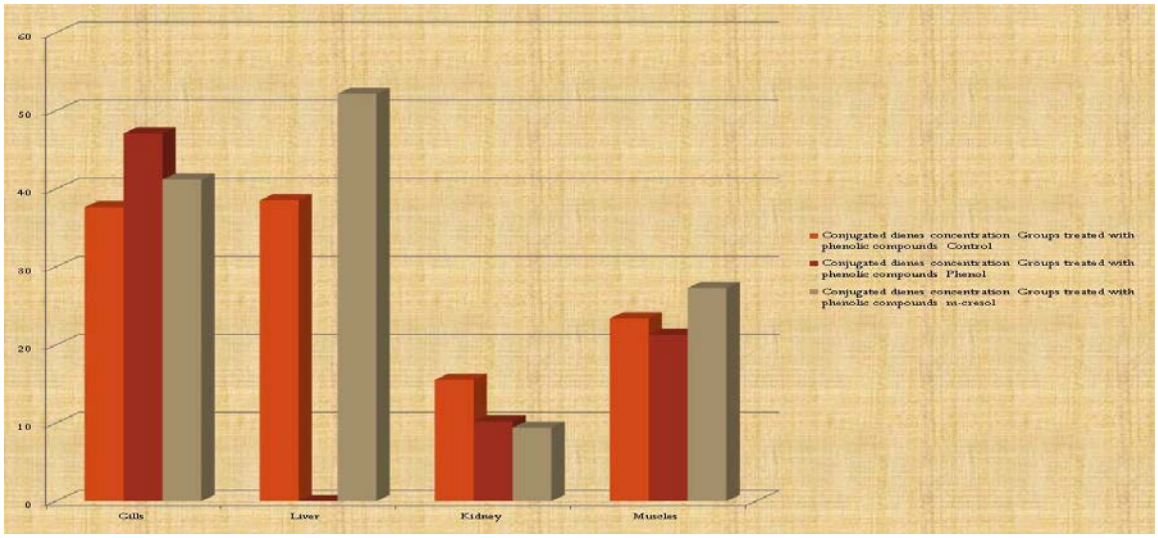

Figure 7: Effect of phenolic compounds on Conjugated dienes in different tissues of L. rohita

Table 8: Effect of phenolic compounds on Hydrogen peroxides in different tissues of *L. rohita*

Tissue	**Hydro-peroxides (HP)**		
	Groups treated with phenolic compounds		
	Control	**Phenol**	**m-cresol**
Gills	13.85±2.75	16.23±2.76	8.34±2.83
Liver	30.34±1.10	40.02±1.10	35.04±1.10
Kidney	15.02±0.25	21.23±0.28	17.56±0.28
Muscles	10±0.24	12.25±034	14.45±0.28

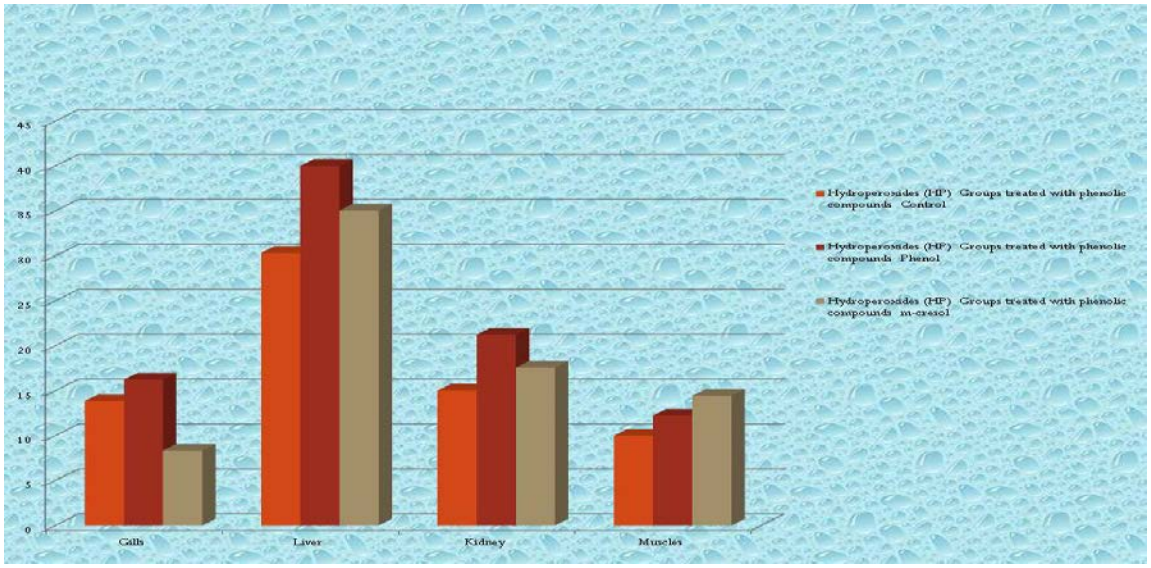

Figure 8: Effect of phenolic compounds on Hydrogen peroxides in different tissues of *L. rohita*