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Research Article

Toxic Effects of Endosulfan on Behaviour, Protein Contents and Antioxidant Enzyme System in Gills, Brain, Liver and Muscle Tissues of Rohu, *Labeo rohita*

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Abstract

The current study was designed to investigate the behavioural and biochemical changes induced by endosulfan in brain, gills, liver and muscle of rohu, *Labeo rohita*. Behavioural changes observed after exposing fish to LC_{50} of endosulfan, were erratic swimming, jumping, loss of equilibrium and balance, hyperactivity, increased air gulping and surface activity. These changes were observed to be more pronounced with length of exposure time. Longer exposure made the fish lethargic, with the lesser operculur beat, adopted a vertical position sometimes and motionless state before dying. Internal hemorrhage was conspicuous. A time dependent decrease in total protein contents and increase in antioxidant enzymes, including catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), glutathione reductase (GR), reduced glutathione (GSH), glutathione peroxidase (GSH-Px) and glutathione-s-transferase (GST) was observed in the brain, gills, liver and muscles of rohu. A time dependent increase in malondialdehyde (MDA) concentration was also observed in different tissues. The current study reports endosulfan as highly toxic to *L. rohita*. Its indiscriminate use should be controlled in order to conserve the population of rohu and other Cyprinids in natural aquatic systems.

Key words: Rohu, fish, endosulfan, behavior, protein contents, LPO, antioxidant enzymes

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Extensive use of pesticides leads to serious health hazards and pollution. It is well established that most of the pesticides induce toxicity and result in severe adverse effects on various biological systems such as the kidneys, liver and muscles etc. when tested on different experimental animals (Ullah and Zorriehzahra, 2015). These effects are either of their modes of action or by producing free radicals, damaging cell components (Khan, 2006). Pesticides act as pro-oxidant, eliciting effects in different organs (Limon-Pacheco and Gonsebatt, 2009). These are most commonly employed in agriculture. These are easily absorbed and are rapidly distributed in different organs. Pesticides interrupt membrane dependent processes such as enzyme activities of different organelles, plasma membrane and nerve conductance (Karaoz *et al*., 2002). Production of reactive oxygen species induces oxidative damage primarily and can damage DNA, proteins and lipids (Ullah *et al*., 2014). Thus, oxidative damage contributes to damage of structural integrity of enzymes, enzymatic activities and can trigger inflammatory processes (Ozyurt *et al*., 2004; Pereira *et al*., 2013).

Whenever there is an imbalance in oxidant to antioxidant ratio, oxidative stress occurs which damages proteins, lipids, nucleic acids and carbohydrates (Rahal *et al*., 2014). Abnormal ROS generation damages cell structure which is a key indication of oxidative damage (Barzilai and Yamamoto, 2004). To cope with oxidative damage and avoid damage from activated ROS, organisms have an antioxidative system such as superoxide dismutase converts superoxide, the parental form of ROS, into hydrogen peroxide which is again converted into water and oxygen by the action of catalase, peroxiredoxin and glutathione peroxidase (Pi *et al*., 2010). Thus, changes in the activities of antioxidant enzymes like catalase, glutathione peroxidase and superoxide dismutase depicts oxidative stress (Ullah, 2015).

ROS are derived from oxygen. These are capable of damaging main biological macromolecule such as proteins, DNA and phospholipids of membranes and lead to cell death (Mates, 2000). Although, the antioxidant defense system can counteract the effects of ROS, still production of ROS in excessive amount can overpower the defense mechanisms which ultimately lead to damage at the cellular level (Ullah *et al*., 2016). This imbalance between the antioxidant defense system and ROS is termed as oxidative stress, resulting in damage of cellular macromolecules (Kelly *et al*., 1998; Al-Ghanim, 2014).

Lipids are oxidized through peroxides formation and this process is called as lipid peroxidation (LPO). The LPO is most widely employed approach in the field of free radical research in aquatic organisms on account of having a higher quantity of lipids and residues of polyunsaturated fatty acid which is a substrate for oxidation (Ullah, 2015). Lipid peroxidation is itself monitored by measuring the end product, malondialdehyde (MDA). It is one of the final products of LPO, hence it is often considered for monitoring its concentration (Lushchak, 2011). The MDA is measured through thiobarbituric acid (TBA). As TBA reacts with many other compounds like carbohydrates, amino acids and various aldehydes, therefore, TBARS (TBA-reactive substances) are referred for measurement of MDA.

Modification in protein contents as a result of ROS has emerged as a key parameter for measuring oxidative stress (Ullah *et al*., 2014). This might be due to the simple spectrophotometric technique of its evaluation with dinitrophenyl hydrazine. As agriculture run offs and industrial effluents lead to water bodies which are then taken up by aquatic organisms, perturbing processes of free radicals (Ullah and Zorriehzahra, 2015). Therefore, the same method is very much employed in aquatic animals for evaluating severity of free radical processes. Protein estimation is a reliable parameter and is recommended for broad usage (Lushchak, 2011).

These pollutants are taken up by aquatic organisms from sediments, food sources, water and suspended particulate matters (Ullah and Zorriehzahra, 2015). The recent advances and current knowledge in general toxicology in general and particularly in hydrobionts toxicology provide a lush arena for aquatic toxicological studies. Moreover aquatic organisms can be used as model organisms for investigating basic processes of damage at the cellular level and free radical protection, tissue injury and finally physiological consequences such as aging or initiation of diseases (Lushchak, 2011).

The current study was aimed to evaluate oxidative stress, behavioural and antioxidative damage induced by endosulfan in *Labeo rohita*, an economically important teleost fish species among Indian major carps. The damage was measured in term of indicators of integrity of antioxidant defense systems, including protein contents, LPO, CAT, POD, SOD, GR, GSH, GSH-Px and GST (Mates, 2000).

MATERIALS AND METHODS

Test animals and their acclimatization: A total of 180 healthy and uniform sized rohu (7.2±1.12 g weight and 9.2±1.35 cm length) fish (*Labeo rohita*-Hamilton, 1822) were distributed in fibreglass circular tanks after conditioning by gradual mixing of water from a circular tank with water in polyethylene bags. Fish were acclimatized before the start of the experiment for ten days. During acclimatization these were fed to satiation

(35% basal protein diet) twice daily at the rate of 5% body weight. Excretory wastes and feed remains were siphoned off every day in order to avoid stress to the fingerlings. Water was changed on a daily basis. During this period temperature (26.5°C), pH (7.5), hardness (300 mg L^{-1}), ammonia (<0.25 ppm) and dissolved oxygen (6-6.8 mg L^{-1}) were checked on a daily basis and efforts were made to keep them in optimum ranges.

Experimental design: The experiment was undertaken in semi-static closed system. Uniform sized healthy fish, regardless of sex, were evenly distributed in six aquaria at a stocking density of 1.5 g L^{-1}. The experiment was carried out in triplicate; first three aquaria served as control while other three as treated group. The LC_{50} of endosulfan for 96 h was observed to be 2.5 μg L^{-1} through problit analysis using semi-static method. The fish in the treatment group were exposed to LC_{50} of endosulfan. The experiment was conducted for 4 days. Behavioural patterns of the fish were monitored keenly. After every 24-96 h, 6 fish from each aquarium were captured before changing water and restoring endosulfan concentration and were anaesthetized with MS222 (60 mg L^{-1}). Their brain, gills, liver and muscles were dissected out on the ice box. The samples were stored at -20°C for further study.

Behavioural study: The behaviour of rohu was keenly observed in both the groups. Their equilibrium, movement, air gulping activity, operculum beat, hyper activeness etc. were studied.

Biochemical study: On account of the small size of liver and brain, three samples from same aquarium were pooled for acquiring requisite weights for biochemical analysis. The total protein content were determined by following Lowry's method (Lowry *et al*., 1951). The LPO level was estimated by following Wright *et al*. (1981). The CAT and POD activities were measured by following Chance and Maehly (1955). The SOD activity was estimated by the method of Kakkar *et al*. (1984). The activity of GR was measured by following Carlberg and Mannervik (1975). The GSH was estimated by the method of Jollow *et al*. (1974). The GSH-Px activity was assayed by the method of Mohandas *et al*. (1984) and GST activity was assayed by following Habig *et al*. (1974).

Statistical analysis: The results expressed as Mean±SE, were analyzed through one way analysis of variances (ANOVA) followed by HSD Tukey test using Statistix Version 8.1. p-value<0.05 was considered as statistically significant.

RESULTS

Different behavioural and morphological changes were observed in rohu after exposure to LC_{50} of endosulfan. These changes in behaviour were observed from 24-96 h. These changes involved jumping, equilibrium loss, rapid swimming, increased air gulping, hyperactivity and increased aggression after 24-48 h of exposure. After 48-96 h, fish became sluggish and operculum beats decreased. At the end of the exposure period fish gathered at aquarium corner and become motionless with adopting vertical positions sometime before dying occasionally. Internal hemorrhage in the brain and change in colour was observed. The morphological and behavioural changes induced in rohu after exposure to endosulfan are given in Table 1.

Endosulfan exposure induced a significant ($p<0.05$) decrease in total protein contents (Table 2) while a significant ($p<0.05$) increase was observed in MDA level in brain, gills, liver and muscles of the treated group as compared to control (Table 3). The activities of CAT (Table 4), POD (Table 5), SOD

Table 1: Behavioural response of *Labeo rohita* exposed to LC_{50} endosulfan

	Treatment groups	
Behaviors	Control	Treated
Jumping	-	+
Hyper activeness	-	+
Equilibrium loss	-	+
Hyperventilation	-	+
Erratic swimming	-	+
Hypo activeness	-	+
Air gulfing	-	+
Internal hemorrhage	-	+
Vertical position	-	+
Motionless state	-	+
Colour change	-	+

+: Present, –: Absent

Table 2: Total protein (mg g^{-1}) content in different tissues at different time intervals

	Brain		Gills		Liver		Muscles	
Time (h)	Control	Treated	Control	Treated	Control	Treated	Control	Treated
24	10.82±0.21^{a}	8.12±0.41^{b}	8.69±0.23^{a}	6.25±0.23^{b}	15.092±0.29^{a}	12.157±0.67^{b}	11.490±0.91^{a}	10.689±0.58^{b}
48	10.69±0.49^{a}	7.42±0.44^{c}	8.82±0.56^{a}	5.82±0.31^{c}	15.191±0.56^{a}	11.123±0.56^{c}	11.423±0.81^{a}	9.889±0.49^{c}
72	10.58±0.41^{a}	7.12±0.59^{c}	8.65±0.78^{a}	5.12±0.56^{cd}	15.958±0.35^{a}	9.445±0.49^{d}	11.723±0.67^{a}	8.188±0.53^{d}
96	10.69±0.67^{a}	6.65±0.51^{d}	8.75±0.45^{a}	4.22±0.61^{d}	15.858±0.21^{a}	8.755±0.69^{e}	11.756±0.59^{a}	7.195±0.47^{e}

Data is represented as Mean±SE (n = 3). Means followed by different letters are significantly different at p<0.05. ANOVA followed by HSD Tukey test

Table 3: Activity of LPO (μmol min^{-1} mg^{-1} protein) in different tissues at different time intervals

	Brain		Gills		Liver		Muscles	
Time (h)	Control	Treated	Control	Treated	Control	Treated	Control	Treated
24	15.41±0.76^{e}	31.41±7.26^{d}	10.12±0.67^{e}	17.24±3.28^{d}	17.74±1.19^{e}	38.14±3.54^{d}	15.53±2.41^{e}	27.49±2.01^{d}
48	15.72±0.67^{e}	41.59±8.64^{c}	10.84±0.61^{e}	24.37±4.17^{c}	17.78±2.41^{e}	51.35±4.78^{c}	15.91±2.05^{e}	39.74±2.12^{c}
72	15.73±0.59^{e}	52.12±4.56^{b}	10.83±0.55^{e}	30.36±2.65^{b}	17.32±2.67^{e}	73.13±6.25^{b}	15.62±3.01^{e}	48.26±3.12^{b}
96	15.71±0.65^{e}	67.18±9.52^{a}	10.43±0.67^{e}	37.01±4.43^{a}	17.29±2.34^{e}	95.07±9.83^{a}	15.71±2.66^{e}	56.83±4.97^{a}

Data is represented as Mean±SE (n = 3). Means followed by different letters are significantly different at p<0.05. ANOVA followed by HSD Tukey test

Table 4: Activity of catalase (μmol min^{-1} mg^{-1} protein) in different tissues at different time intervals

	Brain		Gills		Liver		Muscles	
Time (h)	Control	Treated	Control	Treated	Control	Treated	Control	Treated
24	121.91±19.12^{e}	147.79±16.39^{d}	171.07±12.11^{e}	189.37±12.45^{d}	249.96±14.78^{e}	277.76±11.21^{d}	215.06±19.91^{e}	241.34±11.78^{d}
48	124.13±16.34^{e}	157.18±18.32^{c}	175.89±11.39^{e}	197.72±19.67^{c}	248.59±18.92^{e}	284.85±16.78^{c}	218.72±11.27^{e}	253.89±12.53^{c}
72	124.48±17.29^{e}	163.86±18.17^{b}	173.49±19.25^{e}	209.77±18.38^{b}	251.83±19.17^{e}	299.28±19.56^{b}	217.55±13.32^{e}	269.59±9.67^{b}
96	122.15±22.14^{e}	172.86±15.96^{a}	171.78±10.13^{e}	217.53±21.92^{a}	249.11±16.46^{e}	311.75±23.67^{a}	216.15±13.67^{e}	281.04±15.34^{a}

Data is represented as Mean±SE (n = 3). Means followed by different letters are significantly different at p<0.05. ANOVA followed by HSD Tukey test

Table 5: Activity of POD (μmol min^{-1} mg^{-1} protein) in different tissues at different time intervals

	Brain		Gills		Liver		Muscles	
Time (h)	Control	Treated	Control	Treated	Control	Treated	Control	Treated
24	40.94±5.37^{e}	74.94±11.19^{d}	73.85±14.61^{e}	121.77±12.41^{d}	55.84±9.67^{e}	81.98±11.28^{d}	27.58±9.1^{e}	39.13±8.41^{d}
48	41.64±4.28^{e}	88.29±14.28^{c}	74.43±15.93^{e}	143.17±18.23^{c}	54.89±11.61^{e}	119.18±13.17^{c}	26.09±5.71^{e}	47.62±8.92^{c}
72	40.02±2.79^{e}	101.52±18.34^{b}	74.32±13.56^{e}	177.62±24.27^{b}	54.78±12.76^{e}	139.45±19.01^{b}	26.47±4.91^{e}	59.45±14.01^{b}
96	41.86±3.96^{e}	112.94±13.91^{a}	72.52±11.21^{e}	201.73±45.22^{a}	54.37±10.61^{e}	177.32±24.67^{a}	26.68±6.81^{e}	72.07±15.43^{a}

Data is represented as Mean±SE (n = 3). Means followed by different letters are significantly different at p<0.05. ANOVA followed by HSD Tukey test

Table 6: Activity of SOD (μmol min^{-1} mg^{-1} protein) in different tissues at different time intervals

	Brain		Gills		Liver		Muscles	
Time (h)	Control	Treated	Control	Treated	Control	Treated	Control	Treated
24	121.06±17.22^{e}	151.59±15.89^{d}	135.96±12.23^{e}	172.87±14.20^{d}	159.39±11.65^{e}	179.99±13.12^{d}	132.81±13.81^{e}	151.58±17.29^{d}
48	122.66±12.33^{e}	163.83±19.67^{c}	136.15±14.38^{e}	201.30±18.28^{c}	158.01±12.24^{e}	188.04±16.55^{c}	132.04±16.19^{e}	164.30±14.51^{c}
72	121.31±13.31^{e}	172.12±18.41^{b}	136.22±18.98^{e}	214.71±21.83^{b}	158.76±14.78^{e}	201.16±12.45^{b}	133.66±13.91^{e}	172.34±18.23^{b}
96	121.11±15.34^{e}	181.13±12.98^{a}	134.23±17.67^{e}	221.29±19.74^{a}	159.07±14.61^{e}	214.04±17.19^{a}	132.24±17.14^{e}	189.44±19.81^{a}

Data is represented as Mean±SE (n = 3). Means followed by different letters are significantly different at p<0.05. ANOVA followed by HSD Tukey test

Table 7: Activity of GR (μmol min^{-1} mg^{-1} protein) in different tissues at different time intervals

	Brain		Gills		Liver		Muscles	
Time (h)	Control	Treated	Control	Treated	Control	Treated	Control	Treated
24	1209.93±55.67^{e}	1659.53±39.34^{d}	422.24±41.32^{e}	498.11±19.16^{d}	1393.91±38.52^{e}	1741.24±84.02^{d}	1091.12±61.19^{e}	1621.49±89.91^{d}
48	1207.82±75.89^{e}	2076.34±161.19^{c}	425.92±39.67^{e}	542.11±27.57^{c}	1391.75±43.21^{e}	2201.93±111.72^{c}	1087.97±78.16^{e}	1901.34±86.25^{c}
72	1205.59±35.17^{e}	2503.82±101.45^{b}	421.35±27.91^{e}	681.54±31.23^{b}	1395.04±31.12^{e}	2789.74±128.34^{b}	1089.73±45.28^{e}	2312.71±99.11^{b}
96	1212.55±46.91^{e}	2894.01±123.61^{a}	425.91±32.78^{e}	732.15±44.34^{a}	1392.43±29.21^{e}	3291.78±189.65^{a}	1093.53±56.23^{e}	2521.88±118.32^{a}

Data is represented as Mean±SE (n = 3). Means followed by different letters are significantly different at p<0.05. ANOVA followed by HSD Tukey test

(Table 6), GR (Table 7), GSH (Table 8), GSH-Px (Table 9) and GST (Table 10) in the brain, gills, liver and muscle tissues significantly ($p<0.05$) increased in response to endosulfan exposure.

Table 8: Activity of GSH (μmol g^{-1} tissue) in different tissues at different time intervals

	Brain		Gills		Liver		Muscles	
Time (h)	Control	Treated	Control	Treated	Control	Treated	Control	Treated
24	1.41±0.12^{e}	3.12±0.11^{d}	1.12±0.38^{e}	1.29±0.29^{d}	1.19±0.15^{e}	3.79±0.23^{d}	1.11±0.11^{d}	2.19±0.12^{c}
48	1.44±0.09^{e}	3.87±0.19^{c}	1.09±0.31^{e}	1.38±0.21^{c}	1.20±0.09^{e}	3.91±0.19^{c}	1.13±0.09^{d}	2.61±0.15^{b}
72	1.44±0.16^{e}	4.01±0.21^{b}	1.14±0.22^{e}	1.47±0.27^{b}	1.20±0.14^{e}	4.09±0.21^{b}	1.11±0.14^{d}	2.81±0.21^{ab}
96	1.42±0.11^{e}	4.21±0.18^{a}	1.12±0.19^{e}	1.55±0.19^{a}	1.21±0.12^{e}	4.43±0.33^{a}	1.14±0.18^{d}	2.92±0.11^{a}

Data is represented as Mean±SE (n = 3). Means followed by different letters are significantly different at p<0.05. ANOVA followed by HSD Tukey test

Table 9: Activity of GSH-Px (nmol of GSH oxidized/min/mg protein) in different tissues at different time intervals

	Brain		Gills		Liver		Muscles	
Time (h)	Control	Treated	Control	Treated	Control	Treated	Control	Treated
24	1.19±0.12^{e}	2.24±0.19^{d}	1.63±0.13^{e}	2.21±0.16^{d}	2.11±0.11^{e}	4.21±0.29^{d}	1.66±0.11^{e}	3.22±0.19^{d}
48	1.18±0.11^{e}	2.31±0.11^{c}	1.65±0.11^{e}	2.91±0.21^{c}	2.12±0.15^{e}	4.49±0.21^{c}	1.65±0.09^{e}	3.71±0.21^{c}
72	1.17±0.14^{e}	2.49±0.13^{b}	1.67±0.21^{e}	3.23±0.24^{b}	2.13±0.17^{e}	4.68±0.19^{b}	1.68±0.09^{e}	4.01±0.24^{b}
96	1.19±0.13^{e}	2.71±0.14^{a}	1.69±0.19^{e}	3.67±0.29^{a}	2.11±0.21^{e}	4.79±0.17^{a}	1.67±0.14^{e}	4.33±0.31^{a}

Data is represented as Mean±SE (n = 3). Means followed by different letters are significantly different at p<0.05. ANOVA followed by HSD Tukey test

Table 10: Activity of GST (μmol of chloro-2,4-dinitrobenzyne conjugated formed/min/mg protein)

	Brain		Gills		Liver		Muscles	
Time (h)	Control	Treated	Control	Treated	Control	Treated	Control	Treated
24	1391.30±81.34^{e}	1803.02±83.45^{d}	977.69±69.99^{e}	1101.98±87.14^{d}	1654.23±53.34^{e}	2981.19±115.44^{d}	1271.59±71.92^{e}	2212.01±131.17^{d}
48	1389.97±91.13^{e}	2378.36±103.11^{c}	976.10±79.12^{e}	1361.02±78.89^{c}	1658.32±89.56^{e}	3781.19±106.12^{c}	1269.36±77.79^{e}	2891.02±121.78^{c}
72	1393.31±88.51^{e}	2791.49±113.39^{b}	977.16±81.46^{e}	1521.62±55.34^{b}	1661.20±78.89^{e}	4465.75±197.17^{b}	1273.29±96.17^{e}	3389.22±167.19^{b}
96	1494.16±91.89^{e}	3031.37±141.31^{a}	978.30±74.15^{e}	1765.02±99.62^{a}	1651.91±99.91^{e}	5198.84±545.74^{a}	1269.07±61.69^{e}	4011.53±134.43^{a}

Data is represented as Mean±SE (n = 3). Means followed by different letters are significantly different at p<0.05. ANOVA followed by HSD Tukey test

DISCUSSION

The changes observed in the treated group after exposure to endosulfan were not observed in the control which demonstrates that endosulfan exposure is responsible for these changes. Many investigators also observed a very similar kind of behavioural changes in fish, after exposure to different pesticides such as *Oryzias latipes* (Rice *et al*., 1997), *Silurus glanis* (Koprucu *et al*., 2006), *Cyprinus carpio* (Chebbi and David, 2009) and *Labeo rohita* (Marigoudar *et al*., 2009). Previous studies revealed that severity of response is dependent upon the concentration of pesticide and exposure time. High dose of cypermethrin led to death and coma from weakness, convulsions and respiratory depression (Sandhu and Brar, 2000). In our study, endosulfan treated rohu showed almost the same signs but were sampled before mortality for further study.

The changes in behaviour in the current study as well as those reported in previous studies might be due to the interaction of pesticides with cholinergic neurotransmitter acetylcholine (ACh) (Chandra, 2008; Sharbidre *et al*., 2011). In normal muscle function and behavior, ACh is hydrolyzed by acetylcholinesterase (AChE) enzyme after release into the synaptic cleft and terminates synaptic transmission (Kopecka *et al*., 2004) but endosulfan might have caused neurotoxicity by inhibiting the action of AChE (Bibi *et al*., 2014). Therefore, in its presence, ACh is accumulated in the nerve ending, leading to disrupted nervous activities by overstimulation (Sharbidre *et al*., 2011). The prolonged stimulation of the fibres in the muscles causes paralysis which ultimately results in death (Purves *et al*., 2008). Moreover, the time dependent abrupt and fast swimming as well as lethargy might be due to muscle spasms which in turn might be due to neurologic disruption of nerve function, suffocation and respiratory dysfunction.

Proteins perform different vital functions as functional and structural components of the cells, such as they act as a source of energy during chronic stress conditions and major sources of nitrogen metabolism (Mommsen and Walsh, 1992). The current study revealed that endosulfan exposure resulted in a time dependent decrease in total protein contents in brain, gills, liver and muscles of rohu. Our findings are consistent with the previous studies; many investigators observed similar decrease in total protein contents induced in tissues of different fish species in response to various pesticides such as *Channa striatus* (Tantarpale, 2011), *C. fasciatus* (Singh *et al*., 2010), *Oreochromis niloticus* (Korkmaz *et al*., 2009), *Tor putitora* (Ullah *et al*., 2014) in response to Cypermethrin and *Labeo rohita* in response to fenvalerate (Susan *et al*., 2010), malathion (Patil and David, 2013), indoxacarb (Veeraiah *et al*.,

2013), deltamethrin (Neeraja and Giridhar, 2014) and confidor (Reddy *et al*., 2015). *Lymnaea acuminate*, a freshwater snail, showed a decrease in protein metabolism upon exposure to Carbaryl and Dimethoate pesticides (Tripathi and Singh, 2003).

Endosulfan induced stress condition led to decrease in available oxygen, thus might suppress oxidative metabolism which inhibited energy synthesis (Tripathi and Singh, 2003). Endosulfan might induce hypoxic conditions which lead to respiratory distress on account of lactic acid accumulation (Ullah *et al*., 2014). Therefore, for detoxifying the toxicant and to meet energy requirements for overwhelming endosulfan induced stress, fish changes its respiration to the anaerobic pathway from the aerobic one. As, fish have very little carbohydrate (Rao, 1999), hence protein acts as an alternative source of energy and meets the demanded energy for fish. Consequently, reduced protein content in rohu, in response to endosulfan exposure, might be associated with degradation of protein for meeting higher demanded energy during stress for metabolic purpose or impaired protein synthesis machinery (David *et al*., 2004). The current study revealed a significant higher reduction of protein contents in gills of rohu, followed by liver. This might be on account of direct contact of gills with water, hence endosulfan may possibly flocculate over gill membranes and gill filaments which alter respiration rate (Tiwari *et al*., 2012). It is well established that liver is the main organ of detoxification and metabolism of pesticides, thus liver is more affected as compared to other tissues.

When the defense system of an animal does not neutralize ROS completely. ROS react with membrane lipids and cause oxidative damage (Ahmed *et al*., 2000). So, LPO is thought to be a major outcome of oxidative stress (Hermes-Lima, 2004). The present study revealed a time dependent increase in LPO in response to endosulfan, indicated by an increment in TBARS level. The increase in LPO level in the present study is in congruence to previous studies on *Cyprinus carpio* (Bibi, 2011) and *Oreochromis mossambicus* (Parthasarathy and Joseph, 2011) in response to γ-cyhalothrin. Deltamethrin exposure also increased LPO level in *Channa punctatus* (Atif *et al*., 2005) and *Unio elongatulus euchres* (Koprucu *et al*., 2008). Enhanced LPO was also reported in *Poecilia reticulate* (Sharbidre *et al*., 2011) and *Clarias gariepinus* (Adeyemi *et al*., 2013) in response to diazinon and cypermethrin, respectively.

LPO shows higher affinity for biological membranes rich in polyunsaturated fatty acids. LPO directly affect and decompose double bond of unsaturated fatty acids and ultimately destroy membrane structure (El-Beltagi and Mohamed, 2013), thus causing tissue specific oxidative damage (Adeyemi *et al*., 2013). The current study depicted tissue specific pattern of increase in LPO level and activities of antioxidant enzymes in rohu after exposure to endosulfan. Highest level of LPO and antioxidant enzyme activities were observed in the liver followed by brain and gills. The present result is in correspondence with the findings from previous studies such as deltamethrin and cypermethrin induced highest oxidative damage in liver of *C. punctatus* (Kaur *et al*., 2011) and *C. gariepinus* (Adeyemi *et al*., 2013). The highest elevation in lipid peroxidation in liver might be attributed to excessive ROS production and several provoked redox cycling mechanisms (Reinke *et al*., 1995).

Activities of antioxidant enzymes (AOA) are considered as early warning signs of pollution (Rosety *et al*., 2005). It is well established that pesticides induce oxidative stress and initiate modulation of antioxidant enzymes (Uner *et al*., 2001; Sankar *et al*., 2012). Like other vertebrates, fish have superoxide dismutase as the key antioxidant enzymes for detoxifying superoxide anion radicals. Catalases reduce hydrogen peroxide to oxygen and water. Glutathione peroxidases reduced organic peroxides and hydrogen peroxide by glutathione dependent reaction while glutathione reductases catalyse NADPH dependent glutathione regeneration generated by glutathione peroxidases, from oxidized form (GSSG). Hence, catalase and superoxide dismutase act as first defense line against induced stress (Ullah *et al*., 2014).

In the present study endosulfan exposure resulted in enhanced CAT activity in brain, gills, liver and muscle of rohu. This might be attributed to higher hydrogen peroxide production by the activity of superoxide dismutase which is again detoxified by CAT to water. Similar enhancement of catalase has been reported in *Cyprinus carpio* (Bibi, 2011) and *Tor putitora* (Atika, 2011). Herbicide induced higher CAT activity was observed in *Prochilobus lineatus* (Do Carmo Langiano and Martinez, 2008), *Geophagus brasiliensis* (Filho *et al*., 2001) and *Lepomis macrochirus* (Elia *et al*., 2002) collected from polluted environments. However Malathion exposure decreased CAT activity in *Sparus aurata* (Rosety *et al*., 2005).

Antioxidant enzyme activities in fish depend on pollutant dose, exposure time and more probably on fish species (Elia *et al*., 2002; Nwani *et al*., 2010). These activities are also influenced by habitat and feeding behaviour (Ahmed *et al*., 2000). That's why literature has wide variation in AOA in various tissues of different fish species. In our study activities of GR, POD, GSH, GSH-Px and GST showed a time dependent increase in various tissues of endosulfan exposed fish. These findings are consistent with previous studies, like cypermethrin significantly increased POD, CAT and GR activities in brain, muscle and liver of *C. carpio* (Bibi, 2011),

Labeo rohita (Marigoudar, 2012), *T. putitora* (Ullah *et al*., 2014), malathion in *L. rohita* (Patil and David, 2013), atrazine in *L. macrochirus* (Elia *et al*., 2002) and *C. punctatus* (Nwani *et al*., 2010).

Induction in the activities of glutathione peroxidase depicts a possible defense rendered in response to elevated level of LPO in response to LC_{50} of endosulfan. Glutathione peroxidase plays a vital role against lipid peroxidation, eliminating H_2O_2 and organic compounds. GSH-Px protects membranes from damage induced by the LPO. Its key detoxifying function is terminating radical chain propagation (Van der Oost *et al*., 2003). Our study is in congruence with previous studies on *C. punctatus* (Sayeed *et al*., 2003), *Carassius auratus* (Zhang *et al*., 2004) and *O. niloticus* (Almeida *et al*., 2002).

The GSH level increased in a time dependent manner in brain, gills, liver and muscle tissues of rohu after exposure to endosulfan. It might be attributed to increased supply of GSH which lead to the formation of oxidized glutathione and regeneration of GSH. GSH prevents oxidative stress and detoxify electrophiles (Hasspieler *et al*., 1994; Sies, 1999). During stress the level of GSH might elevate due to increased synthesis. The GSH level decreases, when xenobiotics are removed through direct conjugation via GST or glutathione. Thus, increased level of GSH might be to fulfil the demands of the antioxidant system. Moreover elevation in GSH also indicate exhaustion in biotransformation (phase 2nd), established by increased activity of GST at different time intervals in brain, gills, liver and muscle tissues in response to LC_{50} of endosulfan (Sivaperumal, 2008).

A time dependent elevation was observed in the activity of GST in brain, gills, liver and muscle tissues of rohu. This might be associated with developing a defense mechanism against endosulfan induced stress. GST got increased due to its core role in protecting tissues from oxidative stress induced cellular damage (Banerjee *et al*., 1999; Fournier *et al*., 1992) as it removes extremely reactive electrophiles before they get bind to nucleophilic compounds in the tissues (Sivaperumal, 2008). Furthermore GST is responsible for eliminating and detoxifying xenobiotic and their metabolic products (Jokanovic, 2001). The present study revealed increased activity of GST which is in accordance with previous studies on *L. rohita* (Sivaperumal and Sankar, 2011) and *Brycon cephalus* in response to methyl-parathion (Monteiro *et al*., 2006).

Elevation in the activities of antioxidant enzymes in the present study and previous reported might be attributed to the protective role of antioxidant enzyme pathways for the efficient regulation of ROS formation under stress condition (Ullah *et al*., 2014). This reimbursing response of antioxidant enzymes prevents free radical accumulation and their products in fish under stress condition. Previous studies on pesticides have shown that these are transformed into their respective byproducts having a similar toxicity or occasionally higher toxic potentials than their parental products (Tchounwou *et al*., 2000).

CONCLUSION

The results of the current study revealed that endosulfan is highly toxic to *L. rohita* at acute concentration. The observed altered behaviour, decreased protein contents, increased level of LPO and activities of antioxidant enzymes in brain, gills, liver and muscles of rohu in response to endosulfan confirms the view of using fish as a model organism for toxicological studies as it is very sensitive to toxicants. It is suggested that indiscriminate use of endosulfan should be avoided as it can contribute to the decline of the wild populations of rohu.

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