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Study of Antioxidant Enzyme Activity in Freshwater Catfish Exposed to Lambda-Cyhalothrin

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ABSTRACT

Antioxidant enzymes have been suggested as biological markers of aquatic creatures' exposure to pollutants. Antioxidants are crucial for preserving the redox state of fish tissues and cells, as is widely recognized. Oxidative stress can result from a lack of antioxidants, which can change the animal's physiological state and eventually cause damage to vital tissues. Glutathione peroxidase, catalase, and superoxide dismutase are important enzymes in the antioxidant defence system. In response to oxidative stress, organisms adapt by increasing the activity of antioxidant enzymes. In this study, freshwater catfish exposed to lambda-cyhalothrin will have their antioxidant enzyme activity measured. We measured the superoxide dismutase, catalase, glutathione peroxidase, lipid peroxides, and conjugated diene, activity in the hemolysate. Days 30 and 45 of pyrethroid treatment showed a significant increase in superoxide dismutase activity in fish liver ($P<0.05$), but day 15 showed no discernible change ($P>0.05$). Fish muscle SOD activity increased significantly at greater and lower sub-lethal dosages, respectively; cardiac SOD activity increased significantly overall ($P<0.01$). Catalase activity in the heart tissue of fish from both experimental groups decreased overall in a significant way ($P<0.01$). In fish exposed to pesticides, catalase activity in ovarian tissue increased non-significantly on day 15 ($P>0.05$) and then significantly on days 30 and 45 ($P<0.05$). Fish exposed to pyrethroids showed a significant rise ($P<0.05$) in GPx activity in their brain, heart, and muscle tissues; nevertheless, GPx activity decreased in all three tissues, reversing the previous elevation. The conjugated diene levels in the brain tissue of fish subjected to the greater and lower sub-lethal concentrations of the pyrethroid showed the largest increases, 135.10% and 106.73%, respectively. The lipid hydroperoxide levels in every tissue of the two groups of fish exposed to pyrethroids were found to be significantly ($P<0.01$) elevated.

INTRODUCTION

According to Winston and Diguilio (1991), antioxidant enzymes have been suggested as biological markers of pollution exposure in aquatic animals. According to recent research, free radicals are also responsible for the harmful effects of a variety of environmental pollutants, particularly organophosphates in fish (Hai et al., 1997). Antioxidants are recognized to be crucial for preserving the redox state of fish tissues and cells (Kolayli and Keha, 1999). Oxidative stress can result from a lack of antioxidants, which can change an animal's physiological state and eventually cause damage to vital tissues (Cossu et al., 1997). Important enzymes including glutathione peroxidase, catalase, and superoxide dismutase are part of the antioxidant defense system. Blood peroxide levels are taken as direct indicators of free radical damage (Storey, 1996).

The antioxidant system's enzymes serve as scavengers or protective enzymes, preventing ROS-induced cell damage. Superoxide radicals are eliminated by the antioxidant enzyme superoxide dismutase (SOD). The peroxisomes are where the catalase (CAT) enzyme is mostly found. The enzyme that breaks down hydrogen peroxide is heme-centered (Wilhelm Filho et al., 2000). A selenoenzyme called glutathione peroxidase (GPx) scavenges harmful hydroperoxide molecules from tissues. Free radicals and other foreign substances are destroyed by the thiol transfer process it causes. The organisms' adaptive reaction to oxidative stress is the rise in antioxidant enzyme activity. After being exposed to tetra chlorobiphenyl, rainbow trout and carps have shown increased levels of GPx activity (Otto and Moon, 1995) and to heavy metal copper (Matkovics et al., 1987). Similar elevated levels of antioxidants such as GPx and CAT was reported in the liver tissue of Olive flounder when exposed to phenanthrene (Jee et al., 2004). A significant increase in the antioxidant activities and lipid peroxidation was reported in the liver tissue of *Oreochromis niloticus* and *Cyprinus carpio* exposed to pyrethroid, cypermethin (Uner et al., 2001). *Clarias gariepinus* exposed to petroleum hydrocarbons also showed an elevation in lipid peroxidation and activities of antioxidant

enzymes such as SOD and CAT indicating oxidative stress (Achuba and Osakwe, 2003). An increase in SOD and CAT activities was observed in the heart tissue of rats fed with 7-mg/kg/day dose of lindane (Ananya et al., 2005). In their study of diazonin's impact on lipid peroxidation and antioxidant enzymes in rat heart tissue, Akturk et al. (2006) found that the heart tissue's SOD and CAT enzyme activity significantly increased, indicating accelerated lipid peroxidation. Fish exposed to hexane showed increased lipid peroxidation in their liver tissue, according to Goel et al. (1988). According to reports, heavy metals like cadmium can both increase the formation of ROS and decrease the activity of antioxidant enzymes (Jackim et al., 1970). It is well known that prolonged oxidative stress reduces cell viability, which is commonly measured by LDH leakage from cells (Goswami et al., 2004).

Gupta et al. (2006) reported that methoxychlor inhibits mitochondrial respiration, causes ROS production, and decreases the activity of antioxidant enzymes such as SOD, GPx and CAT in the ovarian fissue of mouse.

Lushchak et al. (2001) observed oxidative stress and antioxidant defenses in the liver muscle and brain tissues of gold fish, *Carassius auratus* during anoxia and reported a significant elevation in the GPy astivity.

The oxidatively damaged end products of lipids are measured by conjugated diene. Rats subjected to a liquid mosquito repellent containing pyrethroids showed markedly higher levels of conjugated diene in their brain and liver tissues (Gupta et al., 1999). The levels of conjugated diene in the brain tissue of gold fish *Carassius auratus*, increased by 75% under anoxia, according to Lushchak et al. (2001). On the other hand, during 20 hours of anoxic submersion, freshwater turtles, Trachemys scripta elegans, showed a 38% drop in hepatic levels of conjugated dienes (Willmore and Storey, 1997).

MATERIALS AND METHODS

Acquisition of Lambda-cyhalothrin, Synthetic pyrethroid; collection, grouping, and maintaining the fresh water female catfish, *Clarias batrachus* for analysis was followed by the method Saravanan et al., (2009).

ASSAY OF ANTIOXIDANT ENZYME ACTIVITY

Assay of Superoxide dismutase (SOD)

Superoxide dismutase was measured using Misra and Fridovich's (1972) methodology. Units/min/mg protein was used to express superoxide dismutase's specific activity. Haemolysate's superoxide dismutase activity was measured in units/mg Hb/min.

Assay of Catalase (CAT)

Catalase assay was done according to the method of Sinha (1972). The specific activity of catalase in the tissue was expressed as micromoles of H2O2 decomposed/minute/mg protein. The specific activity of catalase in the haemolysate was expressed as μ moles of H2O2 consumed/mg Hb/min.

Assay of Glutathione Peroxidase (GPx)

Glutathione peroxidase was done following the method of Rotruck *et al*. (1973). The specific activity of glutathione peroxidase was expressed as microgram of GSH oxidized/min/mg protein. The specific activity of glutathione peroxidase in haemolysate was expressed as μg of GSH oxidized /μg Hb/ minute.

Estimation of Lipid Peroxides (LPO)

Lipid peroxide was estimated in tissues following the procedure of Ohkawa *et al*. (1979). Lipid peroxide content was expressed as nanomoles of TBA reactants formed/mg protein.

Estimation of Lipid Hydroperoxides

Tissue lipid hydroperoxides were estimated by the method of Jiang *et al*. (1991). Lipid hydroperoxide contents are expressed as μmoles of H2O2/mg protein. Lipid peroxide content was expressed as nanomoles of TBA reactants formed/mg protein.

Estimation of conjugated diene

Conjugated diene was measured by the method of Rao and Recknagel (1968). Conjugated diene was expressed as Δ233/mg protein.

RESULTS:

The alterations in the activity of the antioxidant enzyme, superoxide dismutase (SOD) in the ovary, muscle, liver, heart and brain tissues of the fishes exposed to the sub-lethal concentrations of the pyrethroid are tabulated (Table 1a & 1b) .

The liver tissue of fishes of both experimental groups, showed a significant enhancement ($P<0.05$) in the superoxide dismutase activity on the 30th and 45th day of exposure to the pyrethroid, while no significant change ($P>0.05$) was witnessed in the enzyme activity on the 15th day of exposure in both groups of pyrethroid-exposed fishes.

The muscle tissue in fishes from both experimental groups also showed a similar trend of significant increase ($P<0.05$) in comparison to the control fishes. An overall increase of 69.81% and 53.51% was witnessed in the SOD activity of the fishes exposed to the higher and lower sub-lethal concentration of the pyrethroid respectively.

In comparison with the control group of fishes, the ovarian and brain tissues of the pyrethroid-exposed fishes of both groups also showed a similar significant increase ($P<0.05$) in the SOD enzyme activity on the 15th, 30th and 45th day of exposure. A comparison of the enzyme activity in both the experimental group of fishes at different durations of exposure also showed a significant variation ($P<0.05$) in both the tissues.

An overall significant increase ($P<0.01$) was observed in the cardiac SOD activity. However, in comparison with the control group of fishes, both experimental groups showed significant elevation ($P<0.05$) in the SOD activity only on the 30th and 45th day of exposure while no significant variation was witnessed on the 15th day of study.

The changes in the catalase (CAT) enzyme activity of the ovary, muscle, liver, heart and brain tissues of the pyrethroid-exposed fishes are tabulated (Table 2a & 2b). The activity of the catalase enzyme showed a varied response in the tissues under study. The liver and muscle tissue of the fishes exposed to both the sub-lethal concentrations of the pyrethroid showed a significant increase ($P<0.05$) in the catalase enzyme activity when compared to the control group of fishes up to the 30th day of exposure which was followed by a decline in the enzyme activity on the 45th day. However, the activity of the catalase enzyme remained significantly above the control levels in both the tissues.

However, the cardiac tissue of fishes of both experimental groups showed an overall significant decline ($P<0.01$) in the catalase activity. A comparison of the catalase activity of the cardiac tissue at different durations of exposure also showed a significant variation ($P<0.05$). A similar trend of decline was witnessed in the catalase activity of the brain tissue of pyrethroid-exposed fishes in comparison with the control group. While the fishes exposed to the higher sub-lethal concentration of the pyrethroid showed a decline of 88.37% in the catalase activity, the fishes exposed to the lower sub-lethal concentration of the pyrethroid showed an overall decline of 86.91%.

The ovarian tissue of fishes from both experimental groups showed an initial non-significant increase ($P>0.05$) in the catalase activity on the 15th day of the study which was followed by a significant decline ($P<0.05$) in the enzyme activity on the 30th and 45th day of exposure to the pesticide. However, both groups revealed an overall significant change ($P<0.01$) in the enzyme activity after exposure to the sub-lethal concentrations of the pyrethroid.

Table 3a & 3b shows the changes in the glutathione peroxidase (GPx) activity of the fishes exposed to the two different sub-lethal concentrations of the pyrethroid for a period of 45 days.

The liver tissue of fishes from both experimental groups showed a similar pattern of change in the GPx activity. In comparison with the control group a significant increase ($P<0.05$) was witnessed in the GPx activity of the liver tissue of both groups of experimental fishes on the 15^{th} and 30^{th} day of exposure while the 45^{th} day witnessed a decline in GPx activity.

The muscle, heart and brain tissue of fishes from both experimental groups also showed a similar trend of change in the GPx activity where a significant elevation ($P<0.05$) in the GPx activity on the 15th and 30th of exposure to the pyrethroid was followed by a decline in the enzyme activity on the 45th day. A comparison of the GPx activity at different durations of exposure also revealed significant variation ($P<0.05$) in both groups of pyrethroid-exposed fishes. However, in all the tissues the GPx

activity remained significantly elevated (P<0.05) beyond the control levels on the 45th day of exposure.

In fishes exposed to the higher sub-lethal concentration of the pyrethroid, a significant increase (P<0.05) was witnessed in the ovarian GPx activity on the 15th day of exposure which was followed by a significant decline (P<0.05) as witnessed on the 30th and 45th day of exposure to the pyrethroid. Though a similar trend was witnessed in fishes exposed to the lower sub-lethal concentration of the pyrethroid, the increase witnessed in the GPx activity on the 15th day was non-significant (P>0.05) when compared to the control group of animals.

The changes in levels of conjugated diene in the ovary, muscle, liver, heart and brain tissues of the pyrethroid-exposed fishes are tabulated (Table 4a & 4b).

The ovary, muscle, liver, heart and brain tissues of both groups of pyrethroid-exposed fishes showed an overall highly significant increase (P<0.01) in the conjugated diene levels after exposure to the two different sub-lethal concentrations of the pyrethroid, lambda-cyhalothrin. However, the highest increase of 135.10% and 106.73% was observed in the conjugated diene levels of the brain tissue of fishes exposed to the higher and lower sub-lethal concentrations of the pyrethroid respectively. Also a comparison of the conjugated diene levels of the brain tissue at different durations of exposure revealed a significant variation (P<0.05) in both the experimental groups.

The liver and muscle tissue of the pyrethroid-exposed fishes of both groups also showed a significant increase (P<0.05) in the conjugated diene levels when compared to the control group fishes. In the cardiac tissue of fishes exposed to the higher and lower sub-lethal concentration of the pyrethroid an overall significant increase (P<0.01) of 11.93% and 8.13% was witnessed in the levels of conjugated diene after 45 days of exposure to the pyrethroid.

In comparison to the control group of fishes, a significant increase (P<0.05) was also witnessed in the conjugated diene levels of the ovarian tissue on the 15th, 30th and 45th day of exposure in fishes of both experimental groups.

Table 5a & 5b shows the changes in the lipid peroxide content in the various tissues of the fishes exposed to the two different sub-lethal concentrations of the pyrethroid for a period of 45 days.

The liver and muscle tissue of fishes from both experimental groups showed a significant increase (P<0.05) in the lipid peroxide content on the 15th day of exposure to the pyrethroid in comparison to the control group of fishes while the 30th day of pyrethroid exposure showed a decline in the peroxide content which was again followed by a significant elevation (P<0.05) in the peroxide content on the 45th day.

The brain tissue of fishes exposed to the two different sub-lethal concentrations of the pyrethroid, showed a significant (P<0.05) increase in the lipid peroxide content on the 15th day of exposure in comparison to the peroxide content of the control group of fishes. This was again followed by a significant decrease (P<0.05) in the peroxide content on the 30th day and again an increase on the 45th day. A similar trend was also witnessed in the cardiac tissue of the fishes of both experimental groups. The cardiac tissue showed an overall increase of 144.40% and 125% in the lipid peroxide content of the fishes exposed to the higher and lower sub-lethal concentration of the pyrethroid.

On the other hand, the ovarian tissue showed a gradual and significant increase (P<0.05) in the lipid peroxide content throughout the period of exposure. An overall increase of 116.85% and 109.24% was witnessed in the peroxide content after 45 days of exposure. However, all the tissues showed an overall significant increase (P<0.01) in the lipid peroxide content in fishes of both experimental groups on the 45th day of exposure.

Table 6a & 6b shows the changes in the lipid hydroperoxide content in the various tissues of the fishes exposed to the two different sub-lethal concentrations of the pyrethroid for a period of 45 days.

The muscle, liver, heart and brain tissue of the fishes exposed to the two different sub-lethal concentrations of lambda-cyhalothrin showed an initial significant elevation (P<0.05) in the lipid hydroperoxide levels on the 15th day of exposure which was followed by a decline on the 30th day followed by a significant elevation (P<0.05) in the lipid hydroperoxide content on the 45th day of exposure. Also, an overall significant (P<0.01) elevation was witnessed in the lipid hydroperoxide levels of all the tissues of both groups of pyrethroid-exposed fishes.

The ovarian tissue on the other hand showed a gradual and significant increase (P<0.05) in the lipid hydroperoxide content throughout the period of exposure to the pyrethroid in both groups of experimental fishes.

DISCUSSION

Enzymatic antioxidants are the key components of the biochemical machinery that allow for the survival of the lower vertebrate species during anoxia or hypoxia (Hermes-Lima et al., 1998). GPx is more functional than CAT in removal of hydrogen peroxide and is reported to protect the cells against low level oxidant stress. The increased activity of GPx in the present study is in correlation with the decline in the lipid hydoperoxide levels in the various tissues under conditions of oxidative stress since glutathione peroxidase (GPx) is the most important peroxidase for the detoxification of hydroperoxides. In the study of Suman Gulati et al., (2025) synthetic pyrethroid also induced hematological changes.

Gulec et al. (2006) believed that a compensatory mechanism that prevented tissues from being destroyed by toxicants was an increase in SOD enzyme activity, which was secondary to decreased CAT activity. According to Isani et al. (2003), the flux of superoxide radicals that block CAT activity may also be the cause of the lower CAT activity of several tissues in the current investigation. According to Vig and Nemcsok (1989), exposure to severe hypoxia significantly increased *Cyprinus carpio's* liver SOD activity. When exposed to the pyrethroid cypermethrin, the liver tissue of *Oreochromis niloticus* and *Cyprinus carpio* showed a substantial increase in lipid peroxidation and antioxidant activity (Uner et al., 2001).

In the present study, elevation of lipid peroxide and lipid peroxide levels was observed in all the tissues under study at both the sub-lethal concentrations of the pyrethroid. The lipid peroxide levels were reported to be elevated in fish of Ignacio Ramirez reservoir (Mexico) due to the environmental stress caused by the elevated biomagnification of pesticides (Favari et al., 2002).

Mandal and Das (2005) reported a two fold increase in the levels of conjugated diene in the hepatic cells of rats due to CCl4 induction. In the present study, a significant increase was reported in the conjugeted diene levels of the liver, muscle, ovary, heart and brain tissues of fishes subjected to pyrethroid stress when compared to the control animals in response to pyrethroid stress.

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TABLES:

Table 1a: Effect of lambda cyhalothrin at higher sub-lethal concentration (5.768 ppm) on specific activity of superoxide dismutase (SOD) enzyme in various tissues of *Clarias batrachus*

Tissue	F Value	P value	Control	Experimental Days

				15	30	45	Recovery
Ovary	92.09	0.000**	2.79^{a} ± 0.31	3.91^{b} ± 0.22 (+40.14)	4.43^{c} ± 0.27 (+58.79)	5.51^{d} ± 0.28 (+97.49)	3.84^{b} ± 0.15
Muscle	104.96	0.000**	3.81^{a} ± 0.36	4.21^{a} ± 0.25 (+10.49)	5.80^{b} ± 0.22 (+52.23)	6.47^{c} ± 0.26 (+69.81)	4.14^{a} ± 0.29
Liver	68.88	0.000**	6.90^{a} ± 0.23	7.20^{a} ± 0.17 (+4.34)	8.19^{c} ± 0.27 (+18.69)	9.31^{d} ± 0.44 (+34.92)	7.68^{b} ± 0.20
Heart	63.16	0.000**	5.58^{a} ± 0.16	5.93^{ab} ± 0.22 (+6.27)	6.45^{c} ± 0.25 (+15.59)	7.39^{d} ± 0.16 (+32.43)	6.25^{bc} ± 0.24
Brain	234.00	0.000**	0.75^{a} ± 0.12	1.24^{b} ± 0.19 (+65.33)	2.68^{c} ± 0.17 (+257.33)	3.63^{d} ± 0.23 (+384.00)	1.50^{b} ± 0.21

Table 1b: Effect of lambda cyhalothrin at lower sub-lethal concentration (2.884 ppm) on specific activity of superoxide dismutase (SOD) enzyme in various tissues of *Clarias batrachus*

Tissue	F Value	P value	Control	Experimental Days			
				15	30	45	Recovery
Ovary	154.12	0.000**	2.57^{a} ± 0.23	3.34^{b} ± 0.25 (+29.96)	3.97^{c} ± 0.25 (+54.47)	5.52^{d} ± 0.14 (+114.78)	3.44^{b} ± 0.20
Muscle	65.46	0.000**	3.70^{a} ± 0.18	3.90^{a} ± 0.20 (+5.40)	4.44^{b} ± 0.17 (+20.00)	5.68^{c} ± 0.33 (+53.51)	4.06^{ab} ± 0.27
Liver	28.41	0.000**	7.07^{a} ± 0.28	7.15^{a} ± 0.20 (+1.13)	7.92^{b} ± 0.25 (+12.02)	8.27^{b} ± 0.26 (+16.97)	7.45^{a} ± 0.16
Heart	60.60	0.000**	6.00^{a} ± 0.32	6.37^{ab} ± 0.28 (+6.16)	7.42^{c} ± 0.20 (+23.66)	7.92^{d} ± 0.22 (+32.00)	6.76^{b} ± 0.18
Brain	201.26	0.000**	0.74^{a} ± 0.15	1.13^{b} ± 0.19 (+52.70)	2.38^{c} ± 0.15 (+221.62)	3.05^{d} ± 0.18 (+312.16)	1.23^{b} ± 0.16

Table 2a: Effect of lambda cyhalothrin at higher sub-lethal concentration (5.768 ppm) on activity of catalase (CAT) enzyme in various tissues of *Clarias batrachus*

Tissue	F Value	P value	Control	Experimental Days			
				15	30	45	Recovery
Ovary	86.94	0.000**	19.32^{c} ± 0.26	19.75^{c} ± 0.22 (+2.22)	18.15^{b} ± 0.22 (-6.05)	17.17^{a}± 0.29 (-11.12)	18.52^{b} ± 0.32
Muscle	1543.30	0.000**	24.95^{a} ± 0.27	25.68^{b} ± 0.19 (+2.92)	34.23^{e} ± 0.17 (+37.19)	32.37^{d} ± 0.31 (+29.73)	28.72^{c} ± 0.30
Liver	5872.23	0.000**	48.22^{a} ± 0.27	57.31^{c} ± 0.29 (+18.85)	71.87^{e} ± 0.24 (+49.04)	62.33^{d} ± 0.27 (+29.26)	55.34^{b} ± 0.32

Heart	1209.85	0.000**	38.18^{d} ± 0.56	36.34^{c} ± 0.28 (-4.81)	34.54^{b} ± 0.19 (-9.53)	26.65^{a} ± 0.20 (-30.19)	36.66^{c} ± 0.23
Brain	15662.47	0.000**	2.15^{d} ± 0.02	1.16^{c} ± 0.01 (-46.04)	0.25^{b} ± 0.02 (-88.37)	0.19^{a} ± 0.01 (-91.16)	1.18^{a} ± 0.2

Table 2b: Effect of lambda cyhalothrin at lower sub-lethal concentration (2.884 ppm) on activity of catalase (CAT) enzyme in various tissues of *Clarias batrachus*

Tissue	F Value	P value	Control	Experimental Days			
				15	30	45	Recovery
Ovary	20.33	0.000**	19.22^{c} ± 0.15	19.50^{c} ± 0.14 (+1.45)	19.09^{bc} ± 0.31 (-0.67)	18.21^{a} ± 0.31 (-5.25)	18.64^{ab} ± 0.38
Muscle	722.87	0.000**	24.65^{a} ± 0.20	24.94^{a} ± 0.27 (+1.17)	31.31^{d} ± 0.22 (+27.01)	27.40^{c} ± 0.26 (+11.15)	26.16^{b} ± 0.28
Liver	3690.88	0.000**	48.58^{a} ± 0.33	51.30^{b} ± 0.29 (+5.59)	66.05^{d} ± 0.21 (+35.96)	58.29^{c} ± 0.27 (+19.98)	51.68^{b} ± 0.31
Heart	786.91	0.000**	38.33^{d} ± 0.49	37.21^{c} ± 0.22 (-2.92)	35.10^{b} ± 0.20 (-8.42)	29.17^{a} ± 0.27 (-23.89)	37.03^{c} ± 0.33
Brain	13073.95	0.000**	2.14^{e} ± 0.01	1.20^{d} ± 0.02 (-43.93)	0.28^{c} ± 0.02 (-86.91)	0.22^{b} ± 0.02 (-89.71)	1.17^{a} ± 0.10

Table 3a: Effect of lambda cyhalothrin at higher sub-lethal concentration (5.768 ppm) on activity of glutathione peroxidase (GPx) enzyme in various tissues of *Clarias batrachus*

Tissue	F Value	P value	Control	Experimental Days 15	Experimental Days 30	Experimental Days 45	Recovery
Ovary	47.61	0.000**	8.39^{c} ± 0.24	8.95^{d} ± 0.20 (+6.67)	7.96^{b} ± 0.27 (-5.12)	7.12^{a} ± 0.24 (-15.13)	8.38^{c} ± 0.25
Muscle	1036.12	0.000**	10.95^{a} ± 0.24	13.01^{c} ± 0.30 (+18.81)	19.19^{e} ± 0.31 (+75.25)	17.99^{d} ± 0.21 (+64.29)	12.47^{b} ± 0.30
Liver	1092.79	0.000**	15.62^{a} ± 0.21	20.25^{c} ± 0.28 (+29.64)	25.28^{e} ± 0.26 (+61.84)	22.29^{d} ± 0.32 (+42.70)	18.49^{b} ± 0.29
Heart	942.82	0.000**	11.78^{a} ± 0.19	14.71^{c} ± 0.22 (+24.87)	18.32^{e} ± 0.24 (+55.51)	17.12^{d} ± 0.23 (+45.33)	12.52^{b} ± 0.25
Brain	8142.51	0.000**	28.27^{a} ± 0.26	34.16^{b} ± 0.32 (+20.83)	57.15^{d} ± 0.25 (+102.15)	46.21^{c} ± 0.32 (+63.45)	34.65^{b} ± 0.39

Table 3b: Effect of lambda cyhalothrin at lower sub-lethal concentration (2.884 ppm) on activity of glutathione peroxidase (GPx) enzyme in various tissues of *Clarias batrachus*

Tissue	F Value	P value	Control	Experimental Days 15	Experimental Days 30	Experimental Days 45	Recovery
Ovary	11.22	0.000**	8.22^{bc} ± 0.32	8.58^{c} ± 0.16 (+4.37)	8.15^{b} ± 0.21 (-0.85)	7.64^{a} ± 0.21 (-7.05)	8.21^{bc} ± 0.29

Muscle	593.45	0.000**	10.56^{a} ± 0.33	11.74^{b} ± 0.25 (+11.17)	17.16^{d} ± 0.32 (+62.50)	14.29^{c}± 0.28 (+35.32)	10.18^{a} ± 0.29
Liver	751.26	0.000**	15.50^{a} ± 0.29	18.51^{c} ± 0.26 (+19.41)	23.78^{e} ± 0.29 (+53.41)	21.04^{d} ± 0.35 (+35.74)	17.24^{b}± 0.27
Heart	290.52	0.000**	11.89^{a} ± 0.33	13.15^{b} ± 0.19 (+10.50)	16.37^{c} ± 0.31 (+37.67)	15.92^{c} ± 0.28 (+33.89)	12.23^{a} ± 0.36
Brain	1274.26	0.000**	28.18^{a} ± 0.21	30.02^{b}± 0.40 (+6.52)	40.57^{e} ± 0.29 (+43.96)	32.27^{d} ± 0.40 (+14.51)	31.55^{c} ± 0.30

Table 4a: Effect of lambda cyhalothrin at higher sub-lethal concentration (5.768 ppm) on levels of conjugated diene in various tissues of *Clarias batrachus*

Tissue	F Value	P value	Control	Experimental Days (15)	Experimental Days (30)	Experimental Days (45)	Recovery
Ovary	621.11	0.000**	73.555 ± 0.337	78.060 ± 0.284 (+6.12)	79.152 ± 0.281 (+7.60)	80.243 ± 0.208 (+9.09)	74.322 ± 0.331
Muscle	120.81	0.000**	104.97 ± 8.287	120.35 ± 7.895 (+14.65)	159.66 ± 6.564 (+52.10)	188.24 ± 8.227 (+79.32)	113.28 ± 8.301
Liver	132.23	0.000**	191.11 ± 4.579	193.92 ± 5.210 (+1.47)	258.68 ± 7.134 (+35.35)	532.52 ± 6.591 (+178.69)	194.97 ± 0.794
Heart	1717.71	0.000**	92.300 ± 0.227	94.175 ± 0.256 (+2.03)	97.332 ± 0.264 (+5.45)	103.32 ± 0.183 (+11.93)	93.513 ± 0.350
Brain	1944.78	0.000**	226.50 ± 6.680	322.08 ± 6.667 (+42.19)	427.79 ± 6.041 (+88.86)	532.52 ± 6.591 (+135.10)	316.02 ± 6.690

Table 4b: Effect of lambda cyhalothrin at lower sub-lethal concentration (2.884 ppm) on levels of conjugated diene in various tissues of *Clarias batrachus*

Tissue	F Value	P value	Control	Experimental Days (15)	Experimental Days (30)	Experimental Days (45)	Recovery
Ovary	443.31	0.000**	73.118^{a} ± 0.242	74.637^{b} ± 0.328 (+2.07)	76.580^{c} ± 0.227 (+4.73)	79.015^{d} ± 0.239 (+8.06)	74.287^{b}± 0.295
Muscle	75.26	0.000**	104.63^{a} ± 7.305	114.42^{a} ± 8.237 (+9.35)	143.22^{b} ± 6.741 (+36.88)	163.85^{c} ± 6.501 (+56.59)	109.62^{a} ± 6.954
Liver	42811.93	0.000**	192.29^{a} ± 0.251	193.17^{b} ± 0.279 (+0.45)	216.09^{d} ± 0.299 (+12.57)	244.73^{e} ± 0.231 (+27.27)	195.52^{c} ± 0.271
Heart	745.95	0.000**	92.177^{a} ± 0.283	92.938^{b} ± 0.252 (+0.82)	95.747^{c} ± 0.293 (+3.87)	99.677^{d} ± 0.268 (+8.13)	92.758^{b} ± 0.304
Brain	1031.37	0.000**	226.31^{a} ± 6.504	289.45^{b} ± 6.617 (+27.89)	347.59^{c} ± 7.550 (+53.59)	467.87^{d} ± 7.660 (+106.73)	286.38^{b} ± 6.416

Table 5a: Effect of lambda cyhalothrin at higher sub-lethal concentration (5.768 ppm) on Lipid peroxide content in various tissues of *Clarias batrachus*

Tissue	F Value	P value	Control	Experimental Days 15	Experimental Days 30	Experimental Days 45	Recovery
Ovary	422.33	0.000**	$4.45^{a} \pm 0.28$	$6.50^{c} \pm 0.21$ (+46.06)	$7.26^{d} \pm 0.25$ (+63.14)	$9.65^{e} \pm 0.13$ (+116.85)	$5.92^{b} \pm 0.25$
Muscle	400.01	0.000**	$3.28^{a} \pm 0.25$	$6.32^{d} \pm 0.22$ (+92.68)	$4.53^{c} \pm 0.61$ (+38.10)	$8.32^{e} \pm 0.27$ (+153.65)	$3.94^{b} \pm 0.32$
Liver	624.18	0.000**	$3.45^{a} \pm 0.17$	$8.18^{d} \pm 0.26$ (+137.10)	$5.73^{c} \pm 0.27$ (+66.08)	$10.34^{e} \pm 0.24$ (+199.71)	$5.00^{b} \pm 0.36$
Heart	276.64	0.000**	$3.04^{a} \pm 0.29$	$6.34^{d} \pm 0.30$ (+108.55)	$4.87^{c} \pm 0.21$ (+60.19)	$7.43^{e} \pm 0.17$ (+144.40)	$3.53^{b} \pm 0.35$
Brain	5348.35	0.000**	$12.46^{a} \pm 0.20$	$24.16^{d} \pm 0.28$ (+93.90)	$20.28^{c} \pm 0.25$ (+62.76)	$31.44^{e} \pm 0.18$ (+152.32)	$14.70^{b} \pm 0.33$

Table 5b: Effect of lambda cyhalothrin at lower sub-lethal concentration (2.884 ppm) on Lipid peroxide content in various tissues of *Clarias batrachus*

Tissue	F Value	P value	Control	Experimental Days 15	Experimental Days 30	Experimental Days 45	Recovery
Ovary	295.78	0.000**	$4.22^{a} \pm 0.21$	$6.95^{c} \pm 0.26$ (+64.69)	$5.58^{b} \pm 0.23$ (+32.22)	$8.83^{a} \pm 0.24$ (+109.24)	$5.19^{b} \pm 0.32$
Muscle	291.23	0.000**	$3.41^{a} \pm 0.28$	$5.69^{c} \pm 0.23$ (+81.21)	$4.00^{b} \pm 0.22$ (+27.38)	$7.47^{d} \pm 0.19$ (+137.89)	$3.92^{b} \pm 0.31$
Liver	581.33	0.000**	$3.32^{a} \pm 0.26$	$6.93^{c} \pm 0.25$ (+108.73)	$4.48^{b} \pm 0.14$ (+34.93)	$9.36^{d} \pm 0.23$ (+181.92)	$4.22^{b} \pm 0.34$
Heart	207.30	0.000**	$2.84^{a} \pm 0.17$	$6.10^{c} \pm 0.29$ (+114.78)	$4.11^{b} \pm 0.29$ (+44.71)	$6.39^{c} \pm 0.23$ (+125.00)	$3.69^{b} \pm 0.32$
Brain	1589.81	0.000**	$12.51^{a} \pm 0.37$	$19.47^{d} \pm 0.22$ (+55.63)	$16.12^{c} \pm 0.23$ (+28.85)	$24.43^{e} \pm 0.27$ (+95.28)	$14.21^{b} \pm 0.33$

Table 6a: Effect of lambda cyhalothrin at higher sub-lethal concentration (5.768 ppm) on levels of lipid hydroperoxides in various tissues of *Clarias batrachus*

Tissue	F Value	P value	Control	Experimental Days (15)	Experimental Days (30)	Experimental Days (45)	Recovery
Ovary	389.77	0.000^{**}	$90.848^{a} \pm 7.911$	$232.79^{c} \pm 8.477$ (+156.24)	$197.83^{b} \pm 5.469$ (+117.75)	$193.72^{b} \pm 9.030$ (+113.23)	$100.63^{d} \pm 8.021$
Muscle	11034.59	0.000^{**}	$73.452^{a} \pm 0.247$	$102.48^{e} \pm 0.287$ (+39.51)	$87.510^{c} \pm 0.294$ (+19.13)	$94.238^{d} \pm 0.246$ (+28.29)	$80.298^{b} \pm 0.250$
Liver	1213.30	0.000^{**}	$229.56^{a} \pm 6.528$	$527.90^{d} \pm 7.710$ (+129.96)	$418.04^{c} \pm 6.994$ (+82.10)	$423.75^{c} \pm 7.662$ (+84.59)	$371.76^{b} \pm 9.001$
Heart	7967.22	0.000^{**}	$62.237^{a} \pm 0.264$	$86.988^{d} \pm 0.293$ (+39.76)	$75.765^{c} \pm 0.155$ (+21.73)	$86.988^{e} \pm 0.293$ (+39.76)	$71.165^{b} \pm 0.278$

Brain	500.18	0.000**	258.26 ^{a}± 5.119	422.13^{d}±7.502 (+63.45)	403.41^{c}± 10.372 (+56.20)	412.64^{cd}±5.573 (+59.77)	390.66^{b}± 7.289

Table 6b: Effect of lambda cyhalothrin at lower sub-lethal concentration (2.884 ppm) on levels of lipid hydroperoxides in various tissues of *Clarias batrachus*

Tissue	F Value	P value	Control	Experimental Days (15)	Experimental Days (30)	Experimental Days (45)	Recovery
Ovary	224.56	0.000**	112.77^{a}± 8.093	212.23^{d}±6.023 (+88.19)	190.61^{c}±8.072 (+69.02)	230.04^{e}± 8.000 (+103.99)	162.85^{b}± 7.173
Muscle	11133.85	0.000**	78.437 ^{a}± 0.221	102.28^{e}±0.235 (+30.39)	91.450^{c}±0.191 (+16.59)	98.605^{d}± 0.272 (+25.71)	84.225^{b}± 0.218
Liver	565.47	0.000**	317.14 ^{a}± 7.580	524.78^{e}±6.783 (+65.47)	440.23^{c}±9.484 (+38.81)	455.58^{d}±8.772 (+43.65)	390.27^{b}± 7.229
Heart	2694.85	0.000**	69.043 ^{a}± 0.455	83.118^{d}±0.300 (+20.38)	80.530^{c}± 0.277 (+16.63)	86.883^{e}± 0.299 (+25.83)	78.432^{b}± 0.190
Brain	77.91	0.000**	348.26 ^{a}± 7.156	422.27^{c}±9.035 (+21.25)	415.71^{bc}±8.316 (+19.36)	427.60^{c}±11.359 (+22.78)	405.99^{b}± 7.906

P value < 0.01 - ** denotes significance at 1% level (highly significant)
P value 0.011 to 0.05 - * denotes significance at 5% level (significant)
P value > 0.05 – NS denotes non-significant

Different alphabets in means between days in a row denote significance at 5% level.
Means carrying at least one common superscript do not differ significantly (P > 0.05)
Values in parentheses in experimental groups are % reduction (-) or % elevation (+) over control