International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 23s, 2025
https://theaspd.com/index.php

Toxicity Study on Nucleic Acid Dynamics of Synthetic Pyrethroid on Fresh Water Cat Fish Clarias Batrachus

Suman Gulati[1], M. Priyadarssini[2], R. Latha[3], E. Logeswari[4], and K. Revathi[4*]
[1]Vice Principal, Hyderabad, Department of Biochemistry, Sri Venkateshwaraa Medical College Hospital and Research Centre, Ariyur, Puducherry,
[2]Department of Biochemistry, Sri Venkateshwaraa Medical College Hospital and Research Centre, Ariyur, Puducherry,
[3]Department of Physiology, Sri Venkateshwaraa Medical College Hospital and Research Centre, Ariyur, Puducherry,
[4]Research Cell, Sri Venkateshwaraa Medical College Hospital and Research Centre, Ariyur, Puducherry

[*]**Corresponding author: Dr. K. Revathi**
E-mail: reva63@rediffmail.com

ABSTRACT
The credits of pesticides include enhanced economic potential in terms of increased production of food and fiber, and amelioration of vector-borne diseases; their debits have resulted in serious health implications to man and his environment. Fishes major contributors in the field of aquaculture, an important concern today since pesticides hamper the normal physiological processes of the organisms. The growth and the development of the fishes depend on the nucleic acid content (DNA and RNA), which serve as biochemical indices. Cellular enlargement and active protein synthesis are dependent on DNA and RNA content. DNA and RNA dynamics during conditions of stress corresponds to degenerative changes in the tissues leading decreased protein synthesis and cellular proliferation activity since energy is diverted for maintenance of metabolism during stress as evidenced by breakdown and utilization of stored carbohydrates, depletion of protein and lipid. The damage inflicted on the DNA templates, unless repaired, may interfere with replication and transcription resulting in lethal mutations. There has been a dramatic increase in the recent years in the use of pyrethroid pesticides to control insect pests. Owing to the excessive use of synthetic pyrethroids today, the environment and water resources are being rapidly polluted thus endangering aquatic life directly and human life indirectly. Lambda-cyhalothrin is highly toxic to fishes and aquatic invertebrates. However, little is known about the toxicity of sediment associated pyrethroid residues to aquatic organisms despite the agricultural use of these compounds for more than decades. Cat fishes which are extensively grown in rice paddy slurries provide additional source of income to the farmers. In the present study an attempt was made to assess the effect of toxicity in nucleic acid content at two different sub-lethal concentrations of lambda-cyhalothrin on freshwater catfish, Clarias batrachus. The technical grade synthetic pyrethroid, lambda-cyhalothrin with 95% purity used for the study. Healthy Clarias batrachus were chosen and sorted out into three groups Group I, Group II and Groupd III were exposed to the two different sub-lethal concentrations of lambda-cyhalothrin for a period of 45 days. At the end of every of 15, 30 and 45 days blood was collected from five fishes each of the control and test groups. Extraction of total nucleic acids was done; DNA and RNA were estimated. Assay of free radical induced DNA damage was studied and the values were expressed as nanomoles of malondialdehyde formed/hour/mg DNA.
Key words: *Nucleic acid content (DNA&RNA), lambda-cyhalthorin, freshwater fish.*

INTRODUCTION:

Development and growth of fishes depends upon the DNA and RNA content which serve as biochemical indices.[1] Cellular enlargement and active protein synthesis are dependent on DNA and RNA content. A decline in the DNA content of tissues of animals subjected to toxic stress may also be due to inhibition of the enzymes involved in DNA synthesis. More recently, pollution impact is assessed by 'genotoxicity studies' to evaluate the damage caused to fish at molecular level.[2] The synthesis rates of nucleic acids as well as total nucleic acid content contribute to the overall toxicologic assessment. In the present study, the damage was assayed in the control and experimental animals under DNA and RNA levels were estimated and the free radical mediated DNA conditions of pyrethroid-induced stress. DNA and RNA dynamics during conditions of stress corresponds to degenerative changes in the tissues leading to decreased protein synthesis and cellular proliferation activity since energy is diverted for maintenance of metabolism during stress as evidenced by breakdown and utilization of stored carbohydrates, depletion of protein and lipid

reserves The damage inflicted on the DNA templates, unless repaired, may interfere with replication and transcription resulting in lethal mutations.

The decrease in the DNA and RNA content of the liver, muscle, ovary, brain and heart tissues reported in the present study could be attributed to the decreased activity of the HMP shunt key enzyme glucose-6-phosphate dehydrogenase and the decline in the products formed from the non-oxidative phase of the pathway which are required for the synthesis of DNA and RNA The decline in the nucleic acid levels may also be due to the decrease in the ribose-5-phosphate levels which provide the sugar moieties for DNA and RNA as evidenced in the present study by the decrease in the glucose-6-phosphate dehydrogenase activity levels. Thus Lambda-cyhalothrin may interfere with the cell cycle events and this requires further investigation at the molecular level.

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 Gulati et al., (2025).[3]

Extraction of Total Nucleic Acids:

Nucleic acids were extracted from the various tissues following the method of Schneider, (1957).[4] The purified DNA extract thus obtained was used for DNA break studies.

Estimation of DNA:

DNA was estimated by the method of Burton (1956).[5] The DNA content was expressed as mg/g wet tissue.

Estimation of RNA:

RNA was estimated by the method of Schneider (1957).[4] The RNA content was expressed as mg/g wet tissue.

Assay of 5'-nucleotidase:

5'-nucleotidase was assayed by the method of Zekri et al. (1988) and the phosphorous in the supernatant was estimated by the method of Fiske and Subbarow (1925).[6,7] The enzyme activity was expressed as pmoles of phosphorous liberated/min/mg protein.

Assay of free radical induced DNA damage:

DNA break was measured by following the method of Halliwell and Gutteridge (1981).[8] The values are expressed as nanomoles of malondialdehyde formed/hour/mg DNA.

RESULTS:

Effect of Lambda-cyhalothrin on Nucleic Acids:

Table 1a & b shows the changes in the DNA content of the various tissues of the fishes exposed to the two different sub-lethal concentrations of the pyrethroid.

The liver tissue showed a decline of 40.33% and 27.01% in the DNA content after exposure to both the sub-lethal concentrations of the pyrethroid for a period of 45 days. An overall significant decline of ($P<0.01$) was witnessed in the DNA content of the hepatic tissue of the experimental animals of both groups.

On comparison with the control group of animals, the muscle tissue of fishes exposed to the higher sub-lethal concentration of the pyrethroid showed a significant ($P < 0.05$) and continuous decline in the DNA content from the 15^{th} to the 45^{th} day of exposure. However, in fishes exposed to the lower sub-lethal concentration a significant decline ($P < 0.05$) was witnessed only from the 30^{th} day of exposure to the pyrethroid.

The cardiac tissue of fishes exposed to the higher sub-lethal concentration of lambda-cyhalothrin showed a significant decline ($P < 0.05$) in the DNA content on all durations of exposure, while in fishes exposed to the lower sub-lethal concentration of the pyrethroid, though a significant decline ($P < 0.05$) was noticed in the DNA content on the 30^{th} and 45th day of exposure, the 15th day did not show any significant change.

In the brain tissue of fishes exposed to the higher sub-lethal concentration of 5.768 ppm of lambda-cyhalothrin, a significant decline ($P < 0.05$) was witnessed in the DNA content throughout the period of exposure in comparison to the DNA content in the control group of animals. However, a comparison of the DNA content of the brain tissue, at different durations of exposure did not show significant variation ($P > 0.05$). The fishes exposed to the lower sub-lethal concentration of the pyrethroid showed significant decline ($P < 0.05$) in the DNA content only on the 45th day of exposure. However, both groups revealed an overall significant change ($P < 0.01$) in the DNA content of the brain tissue.

The ovarian tissue also showed an overall significant decline ($P < 0.01$) of 39.54% and 36.38% in the DNA content of the fishes exposed to the higher and lower sub-lethal concentration of the pyrethroid respectively. A comparison of the DNA content of the ovarian tissue at different durations of exposure also revealed significant variation ($P < 0.05$) in both groups.

The alterations in the RNA content of the various tissues in the Pyrethroid-exposed fishes are tabulated (Table 2a & 2b).

A significant decline ($P < 0.05$) of 50.35% was witnessed in the RNA content of the hepatic tissue of the fishes after 45 days of exposure to the higher sub-lethal concentration of the pyrethroid. A comparison with the control group of animals also showed significant variation ($P < 0.05$) in the RNA content of the hepatic tissue on the 15^{th}, 30^{th} and 45^{th} day of exposure. A similar trend was witnessed in fishes exposed to the lower sub-lethal concentration of lambda-cyhalothrin with an overall decline of 36.38% in the RNA content after 45 days of exposure.

No significant decline was witnessed in the RNA content of the muscle tissue of fishes from both experimental groups on the 15^{th} day of exposure. However, on 30^{th} and 45^{th} day a significant decline ($P < 0.05$) was witnessed in the RNA content of the muscle tissue of fishes exposed to both the sub-lethal concentration of the pyrethroid.

The ovarian tissue showed an overall significant decline ($P < 0.01$) in the RNA content of the fishes of both experimental groups. While the fishes exposed to the higher sub-lethal concentration of the pesticide showed a decline of 39.65% after 45 days of exposure, the fishes exposed to the lower sub-lethal concentration showed an overall decline of 23.50% in the ovarian RNA content.

The cardiac tissue showed a significant decline ($P < 0.05$) in the RNA content only on the 45th day of exposure in both groups of pyrethroid-exposed fishes. A decline of 42.68% and 27.27% was witnessed in the RNA content of the cardiac tissue of fishes exposed to the higher and lower sub-lethal concentration of the pyrethroid respectively.

The brain also showed an overall significant decline ($P<0.01$) in the RNA levels in fishes of both experimental groups. However, a comparison of the brain RNA content of the pyrethroid-exposed fishes of both groups at different durations of exposure did not reveal any significant variation ($P > 0.05$).

The changes in the 5' nucleotidase activity of the ovary, muscle, liver, heart and brain tissues of the fishes exposed to the sub-lethal concentrations of the pyrethroid are tabulated (Table 3a & 3b).

The hepatic tissue of fishes exposed to the two different sub-lethal concentrations of lambda-cyhalothrin showed a significant decline ($P < 0.05$) in the 5' nucleotidase activity on the 15^{th}, 30^{th}, and 45^{th} day of exposure in comparison to the control group of fishes. A comparison of the enzyme activity at different durations of exposure also showed significant variation ($P < 0.05$) in both groups. A similar trend of significant decline ($P < 0.05$) was witnessed in the 5' nucleotidase activity of the cardiac tissue of both experimental groups.

In the muscle tissue, though fishes exposed to the higher sub-lethal concentration of the pyrethroid showed a significant decline ($P < 0.05$) in the enzyme activity at all durations of exposure, the fishes exposed to the lower sub-lethal concentration showed a significant change ($P < 0.05$) only on the 30^{th} and 45^{th} day of exposure, while no significant decline was witnessed on the 15^{th} day of exposure to the pyrethroid.

The ovarian tissue of fishes of both experimental groups also showed an overall significant decline ($P < 0.01$) in the 5' nucleotidase activity. A decline of 36.88% and 30.75% was found in the enzyme activity after 45 days of exposure to the higher and lower sub-lethal concentration of the pyrethroid respectively.

In comparison to the control group of fishes, the brain tissue of the pyrethroid-exposed fishes of both groups also showed a significant decline ($P < 0.05$) in the enzyme activity.

The changes in the levels of free radical mediated DNA damage in the ovary, muscle, liver, heart and brain tissues of the fishes exposed to the sub-lethal concentrations of the pyrethroid are tabulated (Table 4a & 4b).

There was no significant increase witnessed on the 15th day of exposure in the level of free radical mediated DNA damage in the ovary, muscle, liver and heart tissues of fishes exposed to the two different sub-lethal concentrations of the pyrethroid. However, the 30th and 45th day of exposure showed significant elevation ($P < 0.05$) in the DNA damage in these tissues. However, the brain tissue showed a different trend. A significant elevation ($P < 0.05$) was witnessed in the level of free radical mediated DNA damage of the tissue at all durations of exposure to the pyrethroid. All the tissues of the fishes of both

experimental groups showed an overall significant increase ($P < 0.01$) in the level of free radical mediated DNA damage.

DISCUSSION:

More recently, pollution impact is assessed by 'genotoxicity studies' to evaluate the damage caused to fish at molecular level.[2] Toxic effect of synthetic pyrethroid lambda-cyhalothrin on the functioning of endocrine glands in freshwater catfish, Clarias batrachus was also studied.[9] The synthesis rates of nucleic acids as well as total nucleic acid content contribute to the overall toxicologic assessment. Shea (1987) had also observed disturbances in progression of cell cycle after pesticide intoxication.[10]

Pesticides induce deoxyribonucleic acid damage and structural chromosomal changes Pesticides induce deoxyribonucleic acid damage.[11,12] The intactness of the DNA is the important part of eventual loss of cell structure, proliferation and formation of new tissues and giving rise to mutation and cell death. The DNA:RNA ratio change results in tissue degeneration with a total loss of cellular control mechanisms.[13] Inhibition of the above events is evident from the decrease in the weight of the animal and other organs of the body in the process. Alterations in the DNA structure may be irreversible present study. A decline in the DNA, RNA and DNA/RNA content of the brain, muscle and liver tissues of Clarias batrachus was also reported when the animals were subjected to starvation stress.[14] Altered nucleic acid metabolism was observed in the ovary of arsenic exposed Colisa fasciatus, a fresh water fish.[15]

Inhibition of enzymes that replicate or repair DNA might predispose DNA to damage.[16] Similar mechanisms would have attributed to the nucleic acid damage as witnessed by the increase in the levels of free radical mediated DNA damage assayed in the tissues of pyrethroid-exposed fishes in the present study. The results of this study suggest that the pyrethroid, lambda-cyhalothrin is a potent inhibitor of DNA synthesis even at sub-lethal concentrations, which in turn results in the reduction of RNA level. Anti-AChE compounds attack many enzymes responsible for normal metabolic pathway. Thus, it is possible that in the present study the pyrethroid might have inhibited the enzymes necessary for DNA synthesis.

Gupta et al. (1983) also reported a significant Notopterus notopterus exposed to sub-lethal concentrations of phenolic decline in the 5' nucleotidase activity in the brain and liver tissues of compounds and suggested that the inhibition of this enzyme in the tissues probably represents the uncoupling of oxidative phosphorylation.[17] Similarly, in the present study, exposure of the animals to both the sub-lethal concentrations of the Pyrethroid, lead to a significant decline in the 5' nucleotidase activity of the liver, muscle, ovary, heart and brain tissues.

REFERENCES:

1. Buckley LJ. Changes in ribonucleic acid, deoxyribonucleic acid, and protein content during ontogenesis in winter flounder, Pseudopleuronectes americanus, and effect of starvation. Fish Bull. 1980;77(3):703–708.
2. Abul Farah M, Bushra Ateeq, Niamat Ali M, Waseem Ahmad. Evaluation of genotoxicity of PCP and 2,4-D by micronucleus test in freshwater fish Channa punctatus. Ecotoxicol Environ Saf. 2003;54(1):25-9. doi:10.1016/S0147-6513(02)00037-4.
3. Gulati S, Priyadarssini M, Logeswari E, Revathi K. Effect of lambda-cyhalothrin on the activity of glycolytic pathway and mitochondrial oxidative enzymes. The Bioscan. 2025;20(Suppl 2):873–9. doi: 10.63001/tbs.2025.v20.i02.S2.pp873-879.
4. Schneider WC. Determination of nucleic acids in tissues by pentose analysis. In: Colowick SP, Kaplan WO, editors. Methods in enzymology. New York: Academic Press; 1957. p. 680-4. doi: 10.1016/s0076-6879(57)03442-4.
5. Burton K. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem J. 1956 Feb;62(2):315-23. doi: 10.1042/bj0620315.
6. Zekri M, Harb J, Bernard S, Meflah K. Purification of bovine liver cytosolic 5′-nucleotidase: kinetic and structural studies as compared to the membrane isoenzyme. Eur J Biochem. 1988 Feb;171(1-2):[page numbers]. doi:10.1111/j.1432-1033.1988.tb13860.x.
7. Fiske, Cyrus H., and Yellapragada Subbarow. "The colorimetric determination of phosphorus." J. biol. Chem 66.2 (1925): 375-400.
8. Halliwell B, Gutteridge JM. Formation of a thiobarbituric-acid-reactive substance from deoxyribose in the presence of iron salts: the role of superoxide and hydroxyl radicals. FEBS letters. 1981 Jun 15;128(2):347-52.
9. Saravanan R, Revathi K, Murthy PB. Lambda-cyhalothrin induced alterations in Clarias batrachus. J Environ Biol. 2009 Mar;30(2):265-70. PMID: 20121029.
10. Shea TB. Effect of carbaryl on differentiated and undifferentiated neuroblastoma cell inhibition of growth rates and direct cell toxicity. Bull. Environ. Contam. Toxicology. 1987; 38(1): 143-150
11. Moretti M, Villarini M, Sforzolini GS, Pasquini R. Pesticide-induced primary DNA damage in peripheral blood leukocytes of farm workers evaluated by the computerized 'comet'assay. Biomarkers. 2000 May 1;5(3).
12. Garaj-Vrhovac V, Zeljezic D. Evaluation of DNA damage in workers occupationally exposed to pesticides using single-cell gel electrophoresis (SCGE) assay: pesticide genotoxicity revealed by comet assay. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2000 Sep 20;469(2):279-85.

13. Heath AG. Physiology and ecological health. In: Multiple stresses in ecosystems. 1st ed. Boca Raton: CRC Press; 1998. p. 31. doi:10.1201/9780203746264.
14. Tripathi G, Verma P. Starvation-induced impairment of metabolism in a freshwater catfish. Zeitschrift für Naturforschung C. 2003 Jun 1;58(5-6):446-51.
15. Shukla JP, Pandey K. Altered nucleic acids metabolism in the ovary exposed under arsenic stress in a fresh-water fish, Colisa fasciatus (BL u. SCH). Arch Hydrobiol. 1984;120(2):216. doi:10.1002/aheh.19840120216
16. Aruoma OI, Halliwell B, Gajewski E, Dizdaroglu M. Copper-ion-dependent damage to the bases in DNA in the presence of hydrogen peroxide. Biochem J. 1991 Feb 1;273(Pt 3):601-4. doi: 10.1042/bj2730601. PMID: 1899997; PMCID: PMC1149805.
17. Gupta S, Dalela RC, Saxena PK. Effects of phenolic compounds on 5-nucleotidase activity in some tissues of Notopterus notopterus (Pallas): A biochemical study. Toxicology Letters. 1983;17(1-2):167-73. doi:10.1016/0378-4274(83)90053-X.

TABLES: Table 1a: Effect of lambda-cyhalothrin at higher sub-lethal concentration (5.768 ppm) on DNA levels in various tissues of Clarias batrachus

Tissue	F Value	P value	Control	Experimental Days			
				15	30	45	Recovery
Ovary	706.12	0.000**	$14.49^{e} \pm 0.20$	$13.48^{d} \pm 0.20$ (-6.97)	$9.41^{b} \pm 0.16$ (-35.05)	$8.76^{a} \pm 0.30$ (-39.54)	$13.07^{c} \pm 0.29$
Muscle	60.30	0.000**	$3.30^{d} \pm 0.23$	$2.63^{c} \pm 0.16$ (-20.30)	$2.09^{b} \pm 0.20$ (-36.66)	$1.41^{a} \pm 0.26$ (-57.27)	$2.23^{b} \pm 0.23$
Liver	678.24	0.000**	$13.61^{d} \pm 0.18$	$13.35^{d} \pm 0.21$ (-1.91)	$11.35^{b} \pm 0.20$ (-16.60)	$8.12^{a} \pm 0.27$ (-40.33)	$12.63^{c} \pm 0.19$
Heart	52.86	0.000**	$2.47^{c} \pm 0.22$	$2.03^{b} \pm 0.10$ (-17.81)	$1.31^{a} \pm 0.24$ (-46.96)	$1.00^{a} \pm 0.22$ (-59.51)	$1.89^{b} \pm 0.18$
Brain	14.94	0.000**	$1.50^{c} \pm 0.39$	$0.86^{ab} \pm 0.16$ (-42.66)	$0.52^{a} \pm 0.14$ (-65.33)	$0.54^{a} \pm 0.28$ (-64.00)	$0.936^{ab} \pm 0.24$

Table 1b: Effect of lambda-cyhalothrin at lower sub-lethal concentration (2.884 ppm) on DNA levels in various tissues of Clarias batrachus

Tissue	F Value	P value	Control	Experimental Days			
				15	30	45	Recovery
Ovary	300.29	0.000**	$14.32^{d} \pm 0.26$	$13.90^{d} \pm 0.32$ (-2.93)	$11.09^{b} \pm 0.33$ (-22.55)	$9.11^{a} \pm 0.33$ (-36.38)	$13.09^{c} \pm 0.28$
Muscle	24.52	0.000**	$3.57^{d} \pm 0.24$	$3.26^{cd} \pm 0.31$ (-8.68)	$2.67^{b} \pm 0.28$ (-25.21)	$2.11^{a} \pm 0.28$ (-40.89)	$2.92^{bc} \pm 0.26$

Liver	129.81	0.000**	13.55^{c} ± 0.28	13.38^{c} ± 0.32 (-1.25)	12.36^{b} ± 0.30 (-8.78)	9.89^{a} ± 0.18 (-27.01)	12.27^{b} ± 0.44
Heart	44.21	0.000**	2.59^{d} ± 0.26	2.27^{cd} ± 0.18 (-12.35)	1.72^{b} ± 0.24 (-33.59)	1.08^{a} ± 0.16 (-58.39)	2.02^{bc} ± 0.22
Brain	9.12	0.000**	0.96^{b} ± 0.22	0.97^{b} ± 0.19 (+1.04)	0.57^{a} ± 0.26 (-9.31)	0.43^{a} ± 0.15 (-55.20)	0.63^{a} ± 0.24

Table 2a: Effect of lambda-cyhalothrin at higher sub-lethal concentration (5.768 ppm) on RNA levels in various tissues of Clarias batrachus

Tissue	F Value	P value	Control	Experimental Days			
				15	30	45	Recovery
Ovary	149.01	0.000**	10.49^{d} ± 0.32	9.23^{c} ± 0.34 (-12.01)	8.40^{b} ± 0.34 (-19.92)	6.33^{a} ± 0.30 (-39.65)	9.26^{c} ± 0.23
Muscle	352.98	0.000**	10.17^{d} ± 0.34	10.20^{d} ± 0.32 (+0.29)	5.34^{a} ± 0.29 (-47.49)	6.19^{b} ± 0.27 (-39.13)	8.75^{c} ± 0.24
Liver	307.69	0.000**	9.99^{d} ± 0.33	8.28^{c} ± 0.30 (-17.11)	6.27^{b} ± 0.26 (-37.23)	4.96^{a} ± 0.20 (-50.35)	8.10^{c} ± 0.25
Heart	11.30	0.000**	1.64^{b} ± 0.28	1.50^{b} ± 0.19 (-8.53)	1.47^{b} ± 0.25 (-10.36)	0.94^{a} ± 0.24 (-42.68)	1.02^{a} ± 0.16
Brain	6.226	0.001**	1.00^{b} ± 0.24	0.70^{ab} ± 0.18 (-30.00)	0.65^{a} ± 0.18 (-35.00)	0.46^{a} ± 0.18 (-54.00)	0.64^{a} ± 0.18

Table 2b: Effect of lambda-cyhalothrin at lower sub-lethal concentration (2.884 ppm) on RNA levels in various tissues of Clarias batrachus

Tissue	F Value	P value	Control	Experimental Days			
				15	30	45	Recovery
Ovary	85.35	0.000**	10.68^{d} ± 0.31	9.86^{c} ± 0.29 (-7.67)	9.32^{b} ± 0.26 (-12.73)	8.17^{a} ± 0.15 (-23.50)	9.98^{c} ± 0.19
Muscle	471.35	0.000**	10.19^{d} ± 0.17	10.35^{d} ± 0.28 (+1.57)	7.34^{b} ± 0.24 (-27.96)	5.38^{a} ± 0.18 (-47.20)	8.63^{c} ± 0.27

Liver	182.12	0.000**	9.84^{d} ± 0.22	9.17^{c} ± 0.30 (-6.80)	8.06^{b} ± 0.22 (-18.08)	6.26^{a} ± 0.23 (-36.38)	8.82^{c} ± 0.27
Heart	4.87	0.004**	1.54^{b} ± 0.22	1.40^{ab} ± 0.19 (-9.09)	1.39^{ab} ± 0.20 (-9.74)	1.12^{a} ± 0.23 (-27.27)	1.12^{a} ± 0.20
Brain	3.15	0.03*	0.94^{b} ± 0.24	0.83^{ab} ± 0.20 (-11.70)	0.72^{ab} ± 0.12 (-23.40)	0.61^{a} ± 0.08 (-35.10)	0.72^{ab} ± 0.20

Table 3a: Effect of lambda-cyhalothrin at higher sub-lethal concentration (5.768 ppm) on activity of 5' nucleotidase enzyme in various tissues of Clarias batrachus

Tissue	**F Value**	**P value**	**Control**	**Experimental Days**			
				15	**30**	**45**	**Recovery**
Ovary	106.62	0.000**	8.08^{c} ± 0.29	6.21^{a} ± 0.32 (-23.14)	5.33^{a} ± 0.21 (-34.03)	5.10^{a} ± 0.25 (-36.88)	6.46^{a} ± 0.32
Muscle	169.57	0.000**	5.83^{d} ± 0.33	5.27^{c} ± 0.26 (-9.60)	3.96^{b} ± 0.23 (-32.07)	2.24^{a} ± 0.26 (-61.57)	5.24^{c} ± 0.26
Liver	288.42	0.000**	10.39^{e} ± 0.29	7.16^{c} ± 0.23 (-31.08)	6.35^{b} ± 0.30 (-38.88)	5.52^{a} ± 0.25 (-46.87)	8.50^{d} ± 0.30
Heart	108.85	0.000**	7.28^{e} ± 0.26	6.23^{c} ± 0.30 (-14.42)	5.33^{b} ± 0.29 (-26.78)	4.49^{a} ± 0.23 (-38.32)	6.72^{d} ± 0.22
Brain	52.32	0.000*	2.57^{c} ± 0.15	2.11^{b} ± 0.24 (-17.89)	1.38^{a} ± 0.13 (-46.30)	1.14^{a} ± 0.24 (-55.64)	2.09^{b} ± 0.20

Table 3b: Effect of lambda-cyhalothrin at lower sub-lethal concentration (2.884 ppm) on activity of 5' nucleotidase enzyme in various tissues of Clarias batrachus

Tissue	**F Value**	**P value**	**Control**	**Experimental Days**			
				15	**30**	**45**	**Recovery**
Ovary	67.94	0.000**	8.00^{d} ± 0.31	6.88^{c} ± 0.34 (-14.00)	6.12^{b} ± 0.22 (-23.50)	5.54^{a} ± 0.27 (-30.75)	7.07^{c} ± 0.26

Muscle	83.00	0.000**	5.71^{d} ± 0.30	5.25^{d} ± 0.40 (-8.05)	4.00^{b} ± 0.23 (-29.94)	2.88^{a} ± 0.26 (-49.56)	4.61^{c} ± 0.27
Liver	120.03	0.000**	10.40^{d} ± 0.29	9.52^{c} ± 0.24 (-8.46)	8.30^{b} ± 0.26 (-20.19)	7.27^{a} ± 0.29 (-30.09)	9.10^{c} ± 0.25
Heart	219.17	0.000**	7.56^{e} ± 0.28	6.59^{c} ± 0.22 (-12.83)	6.11^{b} ± 0.27 (-19.17)	4.08^{a} ± 0.16 (-46.03)	7.07^{d} ± 0.14
Brain	16.17	0.000*	2.66^{c} ± 0.31	1.95^{b} ± 0.14 (-26.69)	1.88^{b} ± 0.31 (-29.32)	1.38^{a} ± 0.41 (-48.12)	2.18^{bc} ± 0.14

Table 4a: Free radical mediated DNA damage in various tissues of Clarias batrachus exposed to lambda-cyhalothrin at higher sub-lethal concentration (5.768 ppm)

Tissue	F Value	P value	Control	Experimental Days			
				15	30	45	Recovery
Ovary	23.15	0.000**	250.67^{ab} ± 25.03	238.33^{a} ± 20.35 (-4.92)	304.33^{c} ± 26.79 (-21.40)	351.33^{d} ± 24.66 (+40.15)	286.17^{bc} ± 16.29
Muscle	59.96	0.000**	198.67^{a} ± 11.40	212.67^{a} ± 19.01 (+7.04)	299.67^{c} ± 21.47 (+50.83)	347.67^{d} ± 21.07 (+74.99)	260.67^{b} ± 22.44
Liver	97.56	0.000**	464.17^{a} ± 10.82	475.50^{a} ± 15.11 (+2.44)	549.83^{b} ± 20.69 (+18.45)	632.17^{c} ± 22.27 (+36.19)	457.00^{a} ± 21.36
Heart	93.23	0.000**	321.67^{a} ± 21.41	327.00^{a} ± 16.24 (+1.65)	439.83^{b} ± 20.00 (+36.73)	516.33^{c} ± 23.94 (+60.51)	441.83^{b} ± 23.44
Brain	121.24	0.000*	577.50^{a} ± 16.81	619.67^{b} ± 14.18 (+7.30)	732.00^{c} ± 19.41 (+26.75)	843.50^{d} ± 37.00 (+46.06)	643.67^{b} ± 23.35

Table 4b: Free radical mediated DNA damage in various tissues of Clarias batrachus exposed to lambda-cyhalothrin at lower sub-lethal concentration (2.884 ppm)

Tissue		P value	Control	Experimental Days

	F Value			15	30	45	Recovery
Ovary	6.79	0.000**	246.67[a] ± 22.13	240.67[a] ± 23.07 (-2.43)	285.00[b] ± 21.34 (+15.53)	292.83[b] ± 18.76 (+18.71)	269.67[ab] ± 22.27
Muscle	25.43	0.000**	216.33[a] ± 27.32	216.67[a] ± 24.20 (+0.15)	266.83[b] ± 16.24 (+23.34)	328.50[c] ± 22.26 (+51.85)	247.33[ab] ± 20.85
Liver	27.46	0.000**	464.33[b] ± 21.05	471.17[b] ± 16.41 (+1.47)	484.17[b] ± 13.44 (+4.27)	545.17[c] ± 23.79 (+17.41)	504.50[a] ± 25.67
Heart	48.15	0.000**	324.00[a] ± 21.53	339.83[ab] ± 22.07 (+4.88)	371.67[b] ± 19.52 (+14.71)	464.335[c] ± 17.86 (+43.31)	335.83[a] ± 19.56
Brain	74.30	0.000*	563.67[a] ± 18.25	615.50[b] ± 18.75 (+9.19)	658.17[c] ± 16.09 (+16.76)	743.67[d] ± 19.61 (+31.93)	631.83[bc] ± 21.03