

Erythrocyte Antioxidant And Membrane ATPase Responses To Pyrethroid Pesticide Exposure In Freshwater Catfish

Suman Gulati¹, M. Priyadarssini², K. Revathi^{3*}, E. Logeswari³, R. Latha⁴

¹Vice Principal, Hyderabad, ²Department of Biochemistry, ³Research Cell, ⁴Department of Physiology, Sri Venkateshwaraa Medical College Hospital and Research Centre, Ariyur, Puducherry

*Corresponding author: Dr. K. Revathi

E mail ID: reva63@rediffmail.com

Abstract

Lambda-cyhalothrin is a synthetic pyrethroid that is particularly toxic to fish and other aquatic invertebrates. Despite the fact that pyrethroid residues linked with sediment have been used in agriculture for many years, nothing is known about how harmful they are to aquatic life. Nowadays, the majority of research on oxidative stress in fish evaluates the enzymatic activity in organs including the kidneys, liver, and gills. However, despite the fact that erythrocytes may be more vulnerable to oxidative damage than the majority of other cells, little is known about their antioxidant defense system or systems. It is also known that changes in the permeability of the erythrocyte membrane during oxidative stress might affect the erythrocytes' membrane-bound enzymes. Hence, the aim was to study the toxicological effect of lambda-cyhalothrin on erythrocyte antioxidants and membrane bound ATPases in Freshwater Catfish. Significant biochemical changes were observed in fish erythrocytes following exposure to pyrethroids. By day 30 ($P < 0.05$), superoxide dismutase (SOD) activity had first increased significantly before declining noticeably. Significant decreases in catalase and glutathione peroxidase (GPx) activities were observed by day 45 ($P < 0.01$ and $P < 0.05$, respectively), suggesting weakened antioxidant defense. Furthermore, acetylcholinesterase (AChE) activity decreased dramatically ($P < 0.05$) near the conclusion of the exposure, indicating neurotoxicity. Overall significant decreases ($P < 0.01$) were also observed in membrane-bound Ca^{2+} and Mg^{2+} ATPase activity, indicating compromised ion transport and membrane integrity.

Keywords: Erythrocyte antioxidant, membrane bound ATPases, synthetic pyrethroids

INTRODUCTION

Significant biochemical changes were observed in fish erythrocytes following exposure to pyrethroids. By day 30 ($P < 0.05$), superoxide dismutase (SOD) activity had first increased significantly before declining noticeably. Significant decreases in catalase and glutathione peroxidase (GPx) activities were observed by day 45 ($P < 0.01$ and $P < 0.05$, respectively), suggesting weakened antioxidant defense. Furthermore, acetylcholinesterase (AChE) activity decreased dramatically ($P < 0.05$) near the conclusion of the exposure, indicating neurotoxicity. Overall significant decreases ($P < 0.01$) were also observed in membrane-bound Ca^{2+} and Mg^{2+} ATPase activity, indicating compromised ion transport and membrane integrity

Fish and other aquatic invertebrates are extremely poisoned by the synthetic pyrethroid lambda-cyhalothrin. The synthetic pyrethroid lambda-cyhalothrin affected the male freshwater catfish *Clarias batrachus*'s endocrine glands was studied.¹ Despite the fact that pyrethroid residues linked with sediment have been used in agriculture for many years, nothing is known about how harmful they are to aquatic life. Therefore, an effort was made to investigate the toxicological effects of lambda-cyhalothrin on membrane-bound ATPases and erythrocyte antioxidants in the freshwater catfish *Clarias batrachus*.

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).²

Assay of Erythrocyte Haemolysate Antioxidants and Membrane Bound Enzymes:

Erythrocyte haemolysate was prepared by the method of Lohr and Waller (1963).³

Erythrocyte membrane preparation to study membrane bound enzymes. Erythrocyte membrane were prepared by haemolysing the erythrocytes in distilled water and centrifuging at 20,000 g. Post hemolytic residue was washed twice with 1 mM Tris EDTA (pH 7.4). Erythrocyte membranes were kept frozen at -80°C for assay of Acetyl cholinesterase, $Na^{+}\text{-}K^{+}$ ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase.

RESULTS

The changes in the activity of the erythrocyte haemolysate antioxidants, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) are tabulated (Table 1a & 1b).

The erythrocyte haemolysate antioxidant, superoxide dismutase (SOD) showed a significant increase ($P < 0.05$) in activity up to the 30th of exposure to the pyrethroid which was followed by a significant decline ($P < 0.05$) in the enzyme activity in fishes exposed to the higher sub-lethal concentration of the pyrethroid. A similar trend was also witnessed in fishes exposed to the lower sub-lethal concentration of the pyrethroid. A comparison of the enzyme activity at different durations of exposure to the pyrethroid did not show any significant change between the 30th and 45th day enzyme activity in both groups of pyrethroid-exposed fishes.

The activity of the catalase antioxidant enzyme also showed an overall significant decline ($P < 0.01$) on the 45th day of exposure in both groups of pyrethroid-exposed fishes. In comparison to the control group of animals significant increase ($P < 0.05$) was witnessed in the CAT activity up to the 30th day of exposure to the pyrethroid in both groups of experimental fishes which was followed by a significant decline ($P < 0.05$).

The activity of Glutathione peroxidase (GPx) showed a significant decline ($P < 0.05$) on the 45th day of exposure in fishes exposed to the higher sub-lethal concentration of the pyrethroid. However, when exposed to the lower sub-lethal concentration, no significant change was witnessed in the GPx activity.

Table 2a & 2b shows the changes in the activity of the various erythrocyte membrane bound enzymes in the fishes exposed to the two different sub-lethal concentrations of the pyrethroid for a period of 45 days.

The activity of AChE of the erythrocyte membranes showed a similar trend of change in both groups of pyrethroid-exposed fishes. The initial decline in the AChE activity on the 15th day of exposure was followed by an increase on the 30th day. However, towards the end of the exposure period the erythrocyte AChE activity was found to be significantly declined ($P < 0.05$) in fishes of both experimental groups. However, both groups showed an overall significant change ($P < 0.01$) in the erythrocyte membrane AChE activity.

The erythrocyte membrane bound enzyme, Na⁺-K⁺ ATPase activity showed a significant decline ($P < 0.05$) on the 45th day of exposure in fishes exposed to the two different sub-lethal concentrations of the pyrethroid. The fishes exposed to the higher sub-lethal concentration showed an overall decline of 58.33% in the enzyme activity after 45 days of exposure to the pyrethroid whereas the fishes exposed to the lower sub-lethal concentration showed an overall decline of 36.36%. However, no significant change was witnessed in the enzyme activity on the 15th and 30th day of exposure in fishes of both experimental groups.

The Ca²⁺ ATPase activity of the erythrocytes in both the groups of pyrethroid-exposed fishes showed an overall significant decline ($P < 0.01$). However, in comparison to the control group of fishes, the fishes exposed to the higher sub-lethal concentration of the pyrethroid showed a significant decline ($P < 0.05$) on the 30th and 45th day of exposure while fishes exposed to the lower sub-lethal concentration of the pyrethroid showed a significant decline ($P < 0.05$) in the enzyme activity only on the 45th day of exposure while the 15th and 30th day did not witness any significant change in the ATPase activity.

An overall significant decline ($P < 0.01$) was also witnessed in the erythrocyte membrane bound Mg²⁺ ATPase activity. The fishes exposed to the higher and lower sub-lethal concentration of the pyrethroid showed a decline of 68.75% and 63.15% respectively in the Mg²⁺ ATPase activity after 45 days of exposure to the pyrethroid.

DISCUSSION

Most studies today concerning oxidative stress on fish assess enzymatic activity in organs such as liver, gills and kidneys.⁴ On the other side, little information is available concerning antioxidant defense system(s) of erythrocytes although it is pointed out that erythrocytes are potentially more prone to damage by oxidants than most of the other cells.^{5,6}

Compared to marine species, freshwater species' red blood cells are more sensitive to oxidative stress.⁷ Impact of pyrethroid-induced stress on fish erythrocyte antioxidants, including SOD, CAT, and GPx, was examined in this work. The erythrocyte SOD and CAT activities were seen to exhibit a large increase at the beginning of the experiment and a considerable decrease at the conclusion. Fish exposed to lower sub-lethal concentrations of GPX, the erythrocyte hemolysate, showed a substantial increase in activity in response to the pyrethroid concentration, but fish subjected to greater sub-lethal concentrations showed no significant change in activity across the exposure period.

The notable increase in SOD and CAT levels in the early phases of pyrethroid exposure is a marker of the defense mechanisms required to scavenge oxygen radicals in erythrocytes generated in toxic stress situations,

but the drop in antioxidant levels toward the conclusion of the current study's experimental period might be explained by ROS-induced peroxidation, which increases the fragility and destruction of RBC membranes.⁸

The present findings are in correlation with earlier studies where an enhanced activity of the enzymes of the antioxidant defense system: SOD, GPx and CAT has been reported in rat erythrocytes in response to cadmium intoxication.⁹ A significant elevation in the SOD and CAT levels of the erythrocytes in *Carrasius auratus* after prolonged exposure to cadmium.⁸

The antioxidant enzyme activities of the erythrocytes may decrease as a result of a decrease in glutathione levels brought on by gamma glutamyl cysteine synthetase (gamma-GCS) suppression during oxidative stress, according to studies.¹⁰ The effects of zinc on carp (*Cyprinus carpio*) erythrocytes *in vitro* found that erythrocyte SOD activity did not significantly change, CAT and GPx activity significantly decreased.¹¹ It also suggested that zinc ions might function as a potential cell toxicant, disrupting the antioxidant defense system's functions and changing the characteristics of the erythrocyte membrane, such as increased membrane permeability that causes hemolysis.

CONCLUSION

The present work was useful in obtaining qualitative data on the bio-toxic nature of pyrethroid lambda-cyhalothrin and reveals the need for further investigation at the molecular level to understand. Thus to reduce the environmental impact of pyrethroids such lambda-cyhalothrin, implementation of awareness programmes to farmers regarding the use pattern of these compounds is essential.

REFERENCES

1. Saravanan R, Revathi K, Murthy PB. Lambda cyhalothrin induced alterations in *Clarias batrachus*. J Environ Biol. 2009 Mar;30(2):265-70. PMID: 20121029.
2. Gulati S, Priyadarssini M, Logeswari E, Revathi K. Effect of lambda cyhalothrin on the activity of glycolytic pathway and mitochondrial oxidative enzymes. Bioscan. 2025;20(Suppl 2):873-9. doi:10.63001/tbs.2025.v20.i02.S2.pp873-879
3. G. W. Lohr and H. D. Waller, "Glucose-6-Phosphate Dehydrogenase," In: H. V. Bergmeyer, Ed., Methods of Enzymatic Analysis, Academic Press, New York, 1963, p. 744.
4. Hai DQ, Varga SI, Matkovic B. Organophosphate effects on antioxidant system of carp (*Cyprinus carpio*) and catfish (*Ictalurus nebulosus*). Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology. 1997 May;117(1):83-8. doi:10.1016/S0742-8413(96)00234-4.
5. Soldatov AA, Parfenova IA. The methemoglobin blood level and stability of circulating erythrocytes of the rockfish *Scorpaena porcus* to osmotic shock under conditions of experimental hypoxia. Journal of Evolutionary Biochemistry and Physiology. 2001 Nov;37(6):622-5.
6. Singh M, Sandhir R, Kiran R. Erythrocyte antioxidant enzymes in toxicological evaluation of commonly used organophosphate pesticides. Indian Journal of Experimental Biology. 2006 Jul 1;44(7):580.
7. Kolayli S, Keha E. A comparative study of antioxidant enzyme activities in freshwater and seawater-adapted rainbow trout. Journal of Biochemical and Molecular Toxicology. 1999;13(6):334-7.
8. Zikić RV, Stajin AS, Pavlović SZ, Ognjanović BI, Saičić ZS. Activities of superoxide dismutase and catalase in erythrocytes and plasma transaminases of goldfish (*Carassius auratus gibelio* Bloch.) exposed to cadmium. Physiol Res. 2001 Jan 1;50(1):105-11.
9. Kostić MM, Ognjanović B, Dimitrijević S, Zikić RV, ZSCtajn A, Rosić GL, Zivković RV. Cadmium-induced changes of antioxidant and metabolic status in red blood cells of rats: in vivo effects. European Journal of Haematology. 1993 Aug;51(2):86-92.
10. Rajasekaran NS, Devaraj NS, Devaraj H. Modulation of rat erythrocyte antioxidant defense system by buthionine sulfoximine and its reversal by glutathione monoester therapy. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2004 Mar 2;1688(2):121-9.
11. Akahori A, Gabryelak T, Józwiak Z, Gondko R. Zinc-induced damage to carp (*Cyprinus carpio* L.) erythrocytes in vitro. IUBMB Life. 1999 Jan;47(1):89-98.

TABLES:

Table 1a: Effect of lambda cyhalothrin at higher sub-lethal concentration (5.768 ppm) on erythrocyte haemolysate antioxidants of *Clarias batrachus*

Enzyme	F Value	P value	Control	Experimental Days (Mean ± SD (n=6))			
				15	30	45	Recovery
SOD	94.69	0.000**	2.558 ^c ± 0.088	2.910 ^d ±0.210 (+13.76)	2.965 ^d ± 0.183 (+15.91)	1.468 ^a ± 0.058 (-42.61)	2.082 ^b ± 0.190

CAT	20.48	0.000**	1.080 ^b ± 0.122	1.173 ^{bc} ±0.146 (+8.61)	1.367 ^c ± 0.146 (+26.57)	0.713 ^a ± 0.139 (-33.98)	0.952 ^b ± 0.104
GPx	8.90	0.000**	2.152 ^b ± 0.126	2.218 ^b ±0.095 (+3.06)	2.105 ^b ± 0.158 (-2.18)	1.853 ^a ± 0.101 (-13.89)	2.065 ^b ± 0.068

Table 1b: Effect of lambda cyhalothrin at lower sub-lethal concentration (2.884 ppm) on erythrocyte haemolysate antioxidants of *Clarias batrachus*

Enzyme	F Value	P value	Control	Experimental Days			
				15	30	45	Recovery
SOD	151.78	0.000**	2.562 ^c ± 0.082	2.998 ^d ± 0.193 (+17.01)	3.172 ^d ± 0.124 (+23.80)	1.542 ^a ± 0.108 (-39.81)	2.137 ^b ± 0.125
CAT	31.76	0.000**	1.130 ^b ± 0.131	1.318 ^c ± 0.120 (+16.63)	1.460 ^c ± 0.115 (+29.20)	0.810 ^a ± 0.103 (-28.31)	1.055 ^b ± 0.058
GPx	1.43	0.254 ^{NS}	1.778 ^a ± 0.360	2.115 ^a ± 0.215 (+18.95)	1.990 ^a ± 0.226 (+11.92)	1.937 ^a ± 0.257 (+8.94)	1.922 ^a ± 0.136

Table 2a: Effect of lambda cyhalothrin at higher sub-lethal concentration (5.768 ppm) on erythrocyte membrane bound enzymes of *Clarias batrachus*

Enzyme	F Value	P Value	Control	Experimental Days			
				15	30	45	Recovery
AChE	21.26	0.000**	0.468 ^c ± 0.042	0.317 ^{ab} ±0.056 (-32.26)	0.572 ^d ±0.083 (+22.22)	0.290 ^a ± 0.063 (-38.03)	0.4155 ^{bc} ± 0.052
Na ⁺ K ⁺ ATPase	8.84	0.000**	0.012 ^b ± 0.002	0.010 ^b ±0.002 (-16.66)	0.009 ^b ± 0.003 (-25.00)	0.005 ^a ±0.002 (-58.33)	0.009 ^b ± 0.002
Ca ²⁺ ATPase	18.38	0.000**	0.077 ^c ± 0.004	0.071 ^{bc} ±0.007 (- 7.79)	0.062 ^b ±0.007 (-19.48)	0.053 ^a ± 0.004 (-31.16)	0.073 ^c ± 0.006
Mg ²⁺ ATPase	9.26	0.000**	0.016 ^c ± 0.005	0.014 ^{bc} ±0.00 (-12.50)	0.011 ^{abc} ±0.002 (-31.25)	0.005 ^a ± 0.002 (-68.75)	0.010 ^{ab} ± 0.002

Table 2b: Effect of lambda cyhalothrin at lower sub-lethal concentration (2.884 ppm) on erythrocyte membrane-bound enzymes of *Clarias batrachus*

Enzyme	F Value	P Value	Control	Experimental Days
--------	---------	---------	---------	-------------------

				15	30	45	Recovery
AChE	6.89	0.000**	0.442 ^{bc} ± 0.045	0.373 ^{abc} ± 0.060 (- 15.61)	0.463 ^c ± 0.063 (+4.75)	0.325 ^a ±0.052 (-26.47)	0.357 ^{ab} ± 0.051
Na ⁺ K ⁺ ATPase	7.59	0.000**	0.011 ^b ± 0.001	0.011 ^b ±0.002 (+0.00)	0.009 ^{ab} ±0.002 (-18.18)	0.007 ^a ±0.002 (-36.36)	0.010 ^b ± 0.001
Ca ²⁺ ATPase	5.36	0.002**	0.074 ^b ± 0.006	0.072 ^b ±0.006 (- 2.70)	0.068 ^{ab} ±0.006 (- 8.10)	0.060 ^a ±0.006 (-18.91)	0.075 ^b ± 0.008
Mg ²⁺ ATPase	11.87	0.000**	0.019 ^c ± 0.004	0.015 ^{bc} ± 0.004 (-21.05)	0.012 ^{ab} ±0.002 (-36.84)	0.007 ^a ±0.003 (-63.15)	0.013 ^b ± 0.002

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