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Toxicological Effects of Lambda-cyhalothrin on Liver, Kidney and Testis of Indian Catfish *Clarias batrachus*

Jasdeep Singh[1], Simranjeet Singh[2], Shivika Datta[3], Joydeep Dutta[3], Daljeet Singh Dhanjal[2], Anchal Saini[2] and Joginder Singh[2*]

[1]Department of Bioscience, Barkatullah University, Bhopal, Madhya Pradesh. India.
[2]Department of Biotechnology, Lovely Professional University, Phagwara Punjab; joginder.15005@gmail.com
[3]Department of Zoology, Lovely Professional University, Phagwara Punjab.

Abstract

Aquatic organisms come in direct contact with the pesticides that sprayed onto the agricultural lands are washed out by the rain water. Fishes are affected by exposure to these pesticides. The present study was conducted to determine the toxicity of lambda cyhalothrin on the kidney, liver & testis and biochemical, hematological, behavioural parameters of *Clarias batrachus*. Control group fed with normal diet and kept for 15 and 30 days. The treated groups included normal diet along with 1.33µg L^{-1} of Lambda-cyhalothrin for 15 days and 30 days. The treatment group exhibited disrupted shoaling behaviour characterized by irregular, erratic, and darting swimming movements and loss of equilibrium. Administration of lambda cyhalothrin also causes damage to the tissues of the testes, kidney and liver. Marked changes in the histo-architecture of these organs were observed. All these results suggest that the lambda cyhalothrin induced changes in *C. batrachus* directly or indirectly by modulating the physiological functions in the fishes.

Keywords: Insecticide; Behavioural studies; Histopathology; Haematology; Enzymology

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1. Introduction

The challenge to satisfy the increased demand due to population growth is ever increasing. The task is massive, but Green Revolution in 1960 provided a hope. Main issues related to agriculture development are the attention of public health and proper utilization of natural resources.[1] One of the consequences of technological progress in agricultural has been the release of a large number of chemicals into the environment like chemical pesticides, insecticides and fungicides which are the mainstay in modern agricultural practice. Parkinson and other neurodegenerative disorders, inflammation of the cornea, have been most studied in case of exposure to neurotoxic and carcinogenic hydrocarbons & metals such as Arsenic, Nickel, Mercury etc[2,3,4] and pesticides such as organophosphates, carbamates, organochlorines, pyrethroids and some other insecticides, since they interfere with neurotransmission and function of ion channels in the nervous system [5,6,7,8].

Pesticides sprayed onto the agricultural lands are washed out by the rainwater and gets accumulated in the water bodies. The non-degradable pesticides leave residue in living systems and may rise up in the food chain causing toxic symptoms even to non-target organisms.[9] Various aquatic organisms and fishes being the major part of this ecosystem are at higher risk. The principal toxic effects noted in chronic studies were decreased body weight gain and decreased food consumption.[10]

Even in small quantities, they have adverse effect on various ecological systems. The fact of pesticides had been massively studied with regard to efficacy in pest control and offsite mobility,[11] as they can emigrate from treated fields to air, other land and water bodies.

Lambda-cyhalothrin is a synthetic pyrethroid insecticide used in a variety of applications. They are neurotoxins.[12] These have gained the path to control insects and pests of agriculture and household areas. Annual agricultural use of lambda-cyhalothrin in California has been consistent at approximately 30,000 lbs a.i. per annum from 2000 to 2003 and increased to 40,000 lbs a.i. per annum between 2004 and 2006.

* *Author for correspondence*

[13] In India, Lambdacyhalothrin WP formulation has been recommended for indoor spraying by the national anti-malaria programme and its CS formulation as an insecticide[14]. In addition to its use in vector control such as mosquitoes, it is also highly used in the cotton plantation and in vegetable production. Application rates in field crops typically range from 0.005 to 0.015 kg ai/ha[15]. Aquatic ecosystem plays a major role in life of human beings. Fishes are an important part of aquatic system. They are consumed at large by humans. Also, humans may sometimes use this water for various other needs. So human either directly or indirectly comes in contact with this chemical. In connection with all this, our investigation is framed to explore the toxicological effect of sub-lethal concentration of lambda-cyhalothrin on behavioural, biochemical, histopathological and hematological parameters Indian fish *Clarias batrachus*.

2. Materials and Methods

In the present investigation, we studied the impact of Lambda-cyhalothrin on behavioural, biochemical, hematological parameters and histological changes in the liver, kidney and testis of male Indian fishes *C. batrachus* after different intervals *i.e.*, 15 and 30 days of exposures.

Indian adult fishes *C. batrachus* weighing about 100-150 ± 10 g of body weight were purchased from Local Fish Market, Piplani, Bhopal, (M.P.). They were acclimatized at 27±2°C for 15 days in glass aquariums prior to initiation of the experiment which contained 25 litres of plain potable water (pH 7.54) and fed with normal diet (Tokyo food commercially available). Lambda-cyhalothrin (5% EC) trade name Karate was purchased from Merck India Limited. On the basis of Kumar et al.[16] it was found that the spiked food fed to the fish containing 1.33 µg/L of Lambda-cyhalothrin, tend to induce behavioural changes and dysfunction of certain organs in *Clarias batrachus*.

The experimental fishes were divided into three groups of ten (10) each as follows: **Group-I**: Served as a control group. They were fed with normal diet of 2gm/10 fishes and kept in 25 L of water aquaria for 15 and 30 days; **Group-II**: This group was fed with normal diet of 2gm/10 fishes and kept in 25 L of water aquaria, treated with 1.33µg L^{-1} of Lambda-cyhalothrin for 15 days; **Group-III**: This group was fed with normal diet of 2gm/10 fishes and kept in 25 L of water aquaria, treated with 1.33µg L^{-1} of Lambda-cyhalothrin for 30 days.

All the fishes of the above groups were sacrificed after 24 hours of the last dose according to their dosing patterns along with control fishes. Blood was collected for hematological studies and after that liver, kidney and testis were removed quickly for histological, enzymological and biochemical studies. The body weights were recorded initially and after 15 and 30 days of the experiment; and the behaviour of all experimental fishes was observed initially and also recorded throughout the experiment of 15 and 30 days. Freshly removed liver, kidney and testes were homogenized and following biochemical and enzymological parameters were estimated: Alkaline Phosphatase (ALP) and Acid Phosphatase (ACP) as described by Bergmeyer and Bernt;[17] Glutamate Oxaloacetate Transaminase (GOT) and Glutamate Pyruvate Transaminase (GPT) as described by Reitman and Frankel;[18] Protein estimation as described by Lowry et al.;[19] Creatinine estimation by Alkaline Picrate Method.[20] Freshly collected blood was used for the study of haemoglobin by Sahli's haemoglobinometer and RBCs and WBCs were counted by using a Neubauer's chamber. Liver, kidney and testis were removed, fixed in Bouin's fluid, dehydrated in ethanol series and embedded in paraffin. 5 µm thick paraffin sections were prepared and stained with haematoxylin and eosin.

3. Results

Fishes treated with insecticide showed changes in their body weight. When exposed, they showed a significant decrease in the body weight as compared to the control (Table 1).

Table 1. Body weight (g) of control and pesticide exposed fishes

Groups	Initial Body weight (g)	After experiment (g)
Control	160 ± 2.07	150 ± 2.01
15 Days	169 ± 2.96	137 ± 1.88
30 Days	174 ± 3.35	128 ± 1.45

± = SEM value of five animals

In the present study, the treated group exhibited disrupted shoaling behaviour, localized to the bottom of the test chamber and independence (spreading out) in swimming. In addition, loss of equilibrium and increase in surface activity was also observed. Along with these, colour change was also observed. During the 15 and 30 days of exposure, the fishes become very active and showed

erect movement, the opercular movement increased along with the activity of fishes, while the control fishes showed normal movement and normal behaviour.

The ACP, ALP, GOT, GPT levels were assumed by first calculating the optical density and by applying it was measured in IU/mL. It was seen that GPT and GOT levels in the liver and kidney were significantly increased during the initial period of 15 days and then there was a significant decrease after 30 days of exposure. In contrast the ALP and ACP levels in the hepatic regions showed significant increase in their levels after insecticide exposure of 15 and 30 days with respect to control groups (Table 2).

Due to the exposure of insecticide, the protein level in kidney and liver insignificant increased initially, *i.e.* up to 15 days while, these values lowered in the later part of the experiment up to 30 days as compared to control. In case of the testis, the values of protein content were decreased significantly for 15 and 30 days of exposure. Besides this, creatinine values were seen to be elevated in both kidney and liver after 15 and 30 day exposure to insecticide as compared to control. The elevations were more prominent in kidney as compared to liver (Table 3).

The significant decrease in Haemoglobin (Hb%) was noticed after exposure up to 15 and 30 days in comparison to the control group. The significant decrease in Red Blood Cells were noticed after treatment of insecticide after 15 days in comparison with the control group and the decrease was more significant in 30 day treatment groups as compared to control. The significant increase in White Blood Cell Count was noticed after lambda cyhalothrin treatment group up to 15 days when we compared with controlled group and this increase was more significant in 30 day treatment groups as compared to control (Table 4).

The control section of the liver shows the normal architecture. Large numbers of hepatic cells appeared to be uniformly disposed throughout the specimen. Central vein appeared with normal hepatocytes. A cross section of a lobule had the shape of a polygon, usually a hexagon, the portal triads were typically found at the angles of the polygon (Fig. 1a,b). But with the exposure of the liver to the lambda-cyhalothrin degenerative changes were observed in the liver of 15 days (Fig. 1c). This effect is seen to be more severe in the *later* part of the experiment i.e. after 30 days. The post-treatment changes reported

Table 2. Glutamate oxaloacetate transaminase (GOT), Glutamate pyruvate transaminase (GPT), Acid phosphatase (ACP) and Alkaline phosphatase (ALP) concentration in liver in control and exposed *fishes*

Groups	GOT (IU/mL)	GPT (IU/mL)	ACP (IU/mL)	ALP (IU/mL)
Control	58.33 ± 4.3	121.7 ± 13.5	193 ±14.66	162 ± 9.10
15 Days treated	99.68 ± 9.7	167.0 ± 15.99	217 ± 8.19	193.67 ± 11.7
30 Days treated	78.10 ± 3.2	133.09 ± 11.78	231 ± 11.21	207.06 ± 11.9

± = SEM value of five animals

Table 3. Protein concentration in liver, kidney & testis and Creatinine concentration liver and kidney tissue in control exposed fishes

Groups	Liver tissue	Kidney tissue	Testis tissue	Liver tissue	Kidney tissue
	Protein content (mg/g)			Creatinine content (mg/g)	
Control	17.63 ± 4.3	9.18 ± 2.9	4.03 ± 0.44	0.17 ± 0.03	0.22 ± 0.01
15 Days Treated	23.17 ± 3.5	16.78 ± 3.5	2.47 ± 0.62	0.42 ± 0.08	0.43 ± 0.05
30 Days Treated	11.07 ± 2.41	5.57 ± 0.98	1.97 ± 0.38	0.49 ± 0.04	0.51 ± 0 .08

± = SEM value of five animals

Table 4. Hemoglobin percentage, RBC count and WBC count in blood of control exposed fishes

Groups	HB content (g/dL)	RBC count (10^6/mm^3)	WBC count (10^3/mm)
Control	9.8 ±0.5	4.58 ± 0.27	3.7 ± 0.23
15 Days Treated	7.5 ± 0.32	3.92 ± 0.35	26.4 ± 3.27
30 Days Treated	6.4 ± 0.14	3.18 ± 0.21	29.9 ± 4.23

± = SEM value of five animals

were hepatocytic vacuolation, intercellular vacuolation and infiltration of lymphocytes around the central veins. To a lesser extent, where nuclear death or pycnosis and hepatocytic rupture (Fig. 2a-c).

The kidney of the control group showed normal lymphocytes inter-renal cells, uriniferous tubules (UT) with prominent nuclei. Numerous rounded structures, the malpighian capsules were seen in between the UT. Medullary rays and collecting ducts were also seen in control fishes (Fig. 3a,b).

After treatment, the degenerative changes were observed in the cells of the kidney (Fig. 3c). It showed vacoulisation in uriniferous and inter-renal tubules. These changes were more elevated in 30 days treated fishes with atrophic changes, i.e. reduction in the nuclei and pyknotic nuclei in interrenal cells (Fig. 4a-c).

Section of control testes of male showed normal structure. The seminiferous epithelium was composed of stratified epithelium having different stages of spermatogonial cells. Lumen was filled with large number of spermatozoa. Interstitial cells were also well distributed and showed normal shape and size (Fig.5a,b). Testes examined after the exposure (1.33 µg L^{-1} water) up to 15 and 30 days showed a large number of sperms diminished, almost emptied seminal vesicles. Stroma was somewhat disintegrated. The size of the seminiferous tubules was also diminished, disintegration in seminiferous tubules was also observed. Some Leydig cells were in atrophied condition. Severe degenerative and pycknotic changes in the different stages of developing spermatozoa were observed. Disorganization of the Leydig cells was also seen (Fig. 5c & Fig. 6a-c).

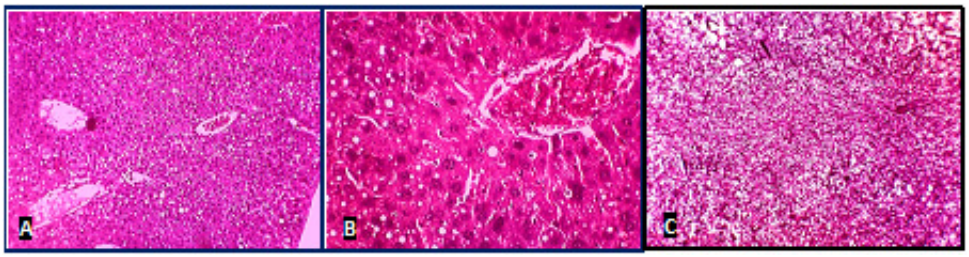


Figure 1. T.S of liver of *Clarias batrachus* (A) control fish showing continuous mass of hepatocytes. The hepatic cells are polygonal in shape with spherical normal hepatic cells with prominent nuclei (H & E x 100). (B) Nucleus and granular cytoplasm with prominent nuclei; hepatic cells are arranged in a sequence (H & E x 400). (C) treated with Lambda cyhalothrin upto 15 days showing hyperactive and degenerative changes in hepatic cells. Hyperactive hepatic cells having fewer amounts of cytoplasmic contents and vacuolization (H & E x 100).

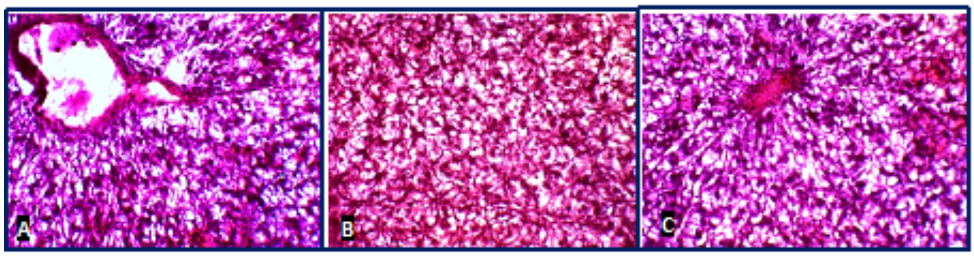


Figure 2. T.S Photomicrograph of liver of fish *Clarias batrachus* (A) treated with Lambda cyhalothrin for 15 days showing hypertrophy in hepatic cells. The maximum cells became enlarged with small nuclei and degenerating hepatic cells (H & E x 400). (B) treated liver showing necrosis, vacuolation, deformed hepatocytes and dilation of extra cellular spaces; the degenerative cells are having pycknotic nuclei after 30 days exposure (H & E x 100). (C) treated liver showing necrosis, distrubed sinusoid and hepatocytes with acentric nuclei after 30 days exposure (H & E x 400).

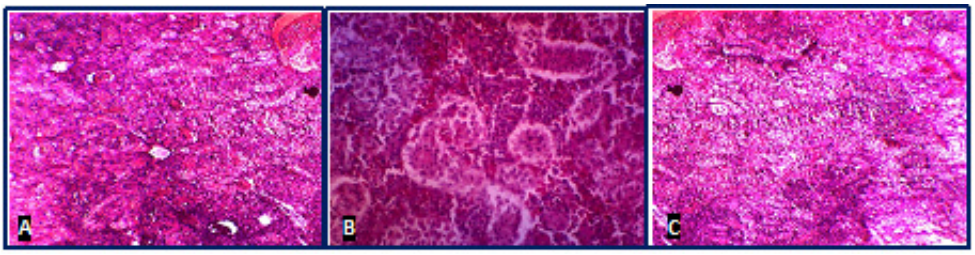


Figure 3. T.S Photomicrograph of normal kidney of *Clarias batrachus* showing histoarchitecture (A) Cellular features of inter renal (INT.R), uriniferous tubules (UT) and glomerulous (H & E x 100). (B) Transverse section depicting cellular features of inter renal (INT.R), uriniferous tubules (UT) and lymphocytic RBCs cells (H & E x 400). (C) Lambda cyhalothrin treated kidney of Clarias batrachus showing degeneration and reduction of glomerulous tissue with vacuolization in renal cells of uriniferous tubules, inter renal cells after 15 days exposure (H & E x 100).

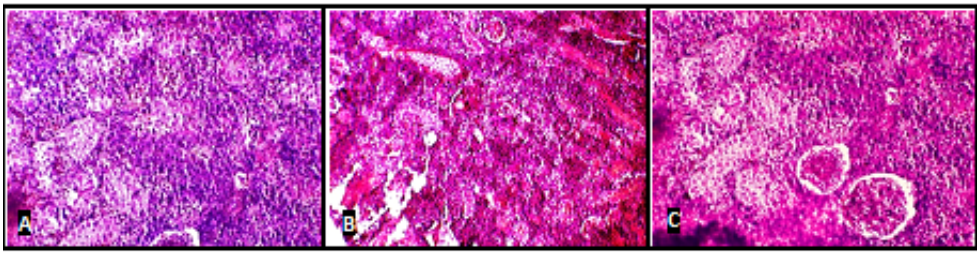


Figure 4. T.S Photomicrograph of 15 days exposed Lambdacyhalothrin kidney of *Clarias batrachus* (A) showing degeneration and reduction of glomerulous tissue and less amount of cytoplasmic material with vacuolization of renal cells of uriniferous tubules, inter renal cells and lumen is slightly enlarged (H & E x 400). (B) The Photomicrograph of 30 days exposed Lambdacyhalothrin kidney of *Clarias batrachus*, showing hypertrophy, severe degeneration in uriniferous tubules and severe cellular disintegration (H & E x 100). (C) The Photomicrograph of 30 days exposed Lambdacyhalothrin kidney of *Clarias batrachus*, showing hypertrophy, severe degeneration in uriniferous tubules and severe cellular disintegration (H & E x 400).

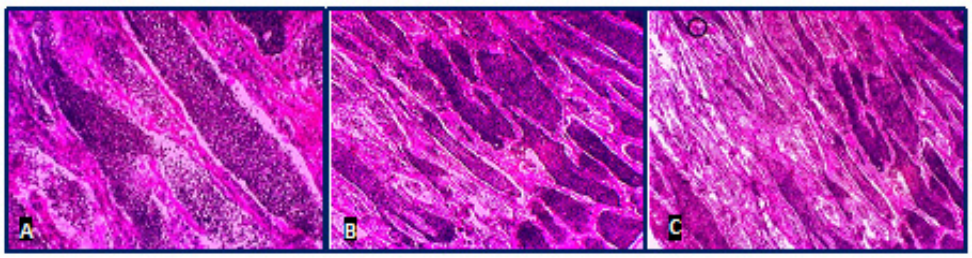


Figure 5. Transverse section of control testis of Clarius batrachus (A) The seminiferous epithelium composed of stratified epithelium having different stages of spermatogonial cells. Lumen filled with large number of spermatozoa. Interstitial cells are also well distributed and showed normal shape and size (H&E 100X). (B) The seminiferous epithelium composed of stratified epithelium having different stages of spermatogonial cells. Lumen filled with large number of spermatozoa (H&E 400X). (C) The Photomicrograph of 15 days exposed Testis showing number of sperm diminished almost emptied seminal vesicles. Stroma is somewhat disintegrated. Size of the seminiferous tubules diminished (H&E 100X).

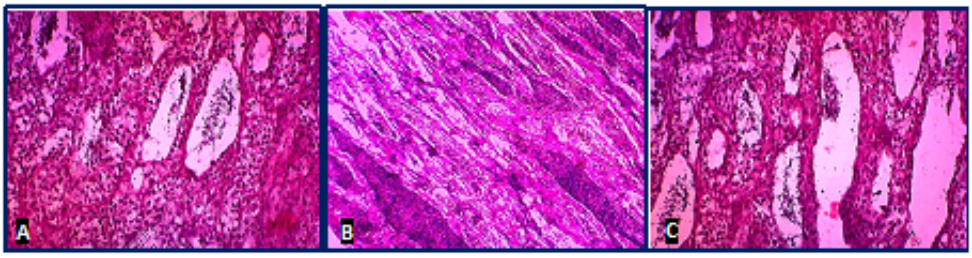


Figure 6. T.S Photomicrograph of testis (A) exposed for 15 days with Lambdacyhalothrin showing the number of sperm diminished almost emptied seminal vesicles. Stroma is some that disintegrated. The size of the seminiferous tubules diminished (H&E 400X). (B) T.S of testis examined after Lambdacyhalothrin exposure up to 30 days showing diminished stroma empty seminiferous tubules, depiction of severe tissue damage and empty lumen. Degenerative pycknotic and Leydig cells are also seen Number of tubules lesser. Hypotrophied seminal vesicles whereas the stroma has extended. (H&E 100X). (C) Testis examined after Lambdacyhalothrin exposure up to 30 days in the transverse section showing diminished stroma, empty seminiferous tubules, depiction of severe tissue damage and empty lumen. Degenerative pycknotic and Leydig cells are also seen Number of tubules lesser. Hypotrophied seminal vesicles whereas the stroma has extended. (H&E 400X).

4. Discussion

Lambda-cyhalothrin is a well known neurotoxin. They have a high rate of gill absorption, which in turn would contribute in the sensitivity of fish to aqueous pyrethroid exposures. Fish seems to be deficient in the enzyme and their metabolism, in fish is largely oxidative.[21] Doses of pesticide which donot cause mortality in fish still cause significant changes in behaviour and physiology that impair both survival and reproduction.[22] In our study, we observed the behavioural, hematological, enzymological, biochemical parameters and histopathological alterations. In addition, loss of equilibrium and increase in surface activity were also observed. Along with these, color change was also observed. During the 15 and 30 days of exposure of lambda cyhalothrin, the fishes became very active and showed erect movement, may be due the accumulation of acetylcholine at cholinergic synapses ensuring hyperstimulation,[23] the opercular movement increased along with the activity of fishes, while the control fishes showed normal movement and normal behaviour. Similar types of results were observed by Kumar et al.[16] The later phase of exposure results in loss in the muscular activity and equilibrium due to decreased acetylcholinestrase activity.

A significant decrease in Hb% was noticed in *C. batrachus* after treatment up to 15 and 30 days. It may be due to suppression or alteration in haemopoiesis in bone marrow. A quiet percentage reduction in total erythrocytes is noticed in our study, which revealed that *C. batrachus* exposed became anemic, possibly due to hemodilution resulting from impaired osmoregulation across the gill epithelium.[24] A similar reduction was observed in the erythrocytes of *Nile tilapia* exposed to pesticides.[25]

In present study leukocyte count were significantly increased after 15 days' insecticide exposure in *C. batrachus*. These values increased more significantly in the *later* part of the experiment, i.e. 30 days as compared to control group. An increase in the total leukocyte count may be due to several factors like increase in thrombocytes, lymphocyte or squeezing of WBC in peripheral blood.[26,27] An increase in the TLC could be due to stimulated lymphopoiesis or enhanced release of lymphocytes from lymphomyeloid tissues.[28]

The liver and kidney are the rich sources of both GOT and GPT enzymes. Any damage to the liver and kidney cells will result in the increase in both of these enzymes.[29] In our study, we found increased levels of GOT and GPT in both kidney and liver with the exposure of insecticide for 15 days of experiment. However, GOT and GPT inhibition was observed in later part of the experiment i.e. 30 days as compared to control. Our finding are in agreement with Oruç and Uner[30] who reported inhibition of GPT and GOT enzyme activities following 20 and 30 days of exposure of 2, 4-Diamin to *Cyprinus carpio*. Sadhu et al.[31] also reported inhibition of GOT and GPT activities in the serum of *Channa striatus* following exposure to malathion for 10 days.

In present study significant increase were observed in ALP and ACP activities in liver and kidney after lambda-cyhalothrin exposure for 15 and 30 days, this increase was duration dependent. Our results inferred with the findings of Al-Attar[32] who also reported significant elevations of ALP after exposure to cadmium and attributed the increase to liver dysfunction.

The exposure of sublethal concentrations of lambda cyhalothrin in our experiment on male reproductive organ i.e. testis of fish *Clarius batrachus*, caused elevation in the ACP and ALP levels after 15 days exposure and the increase were more prominent after 30 days exposure as compared to control. This may be due to tissue injury. However, the increased activity of ACP and ALP, indicated the pathological destructive process.[33]

The hepatic and renal protein levels were significantly increased after insecticide exposure for 15 days, whereas significantly decreased in the *later* part of the experiment as compared to control. It might be due to catabolism of protein and malfunction of liver.[34] The changes in the levels of protein with treatment suggest either an increased catabolism of bio molecules to meet stress or their reduced synthesis due to impaired tissue.[35]

Gill et al.[36] found an increase in protein level following Endosulphan intoxication in the liver of *Barbus conchonious*. They also concluded that increased production may be due to the compensatory production of enzymes lost as a result of tissue necrosis or to meet increased demand to detoxify the pesticides. The quantity of protein may also be affected due to impaired incorporation of amino acids into polypeptide chains.[37]

In addition, it has been also observed that lambda cyhalothrin significantly decreases the concentration of protein in fish testis for 15 and 30 days' exposure as compared to control. This may be because of disruption of the protein synthesis or lack of necessary enzymes required for protein synthesis. The changes were also noticed by Kumar et al.[38]

Creatinine is the catabolic product of creatinine phosphate, which is used by the skeletal muscle. Our study showed that creatinine level was increased in *C. batrachus* after exposure for 15 days. This decrease was more prominent in the *later* part of the experiment i.e. 30 days.

The liver of fishes exposed to insecticide for 15 and 30 days revealed, the congestion of hepatic blood vessels, sinusoids and vascular degeneration with nuclear changes. In some areas the lesions were diffused and the hepatic cells showed dissociation disorganization, mononuclear cell infiltration, enlargement of the veins, and increase in the number of Kupffer cells and cytoplasmic vacuolization. Similar results were observed by Binukumar and Gill.[39] These findings were also noticed by Lakkawar et al.[40]; these changes may be due to necrosis and morphological changes associated with death of liver cells.[41]

The kidney of lambda cyhalothrin exposed fishes over 15 days showed congestion of renal blood vessels in both cortex and medulla together with perivascular edema. Shrinkage of some glomerular tuft due to edema of Bowman's capsule was also observed. Cystic dilation, degeneration and coagulative necrosis were seen in some renal tubules. These degenerative changes were more severe in the later part of the experiment, i.e. 30 days as compared to control. Similar changes were also observed by Tayebati et al.[42]

In addition, Testis histo-architecture examined after lambda cyhalothrin exposure (1.33 μg L^{-1}) up to 15 days showed higher diminished stroma, decrease in sperm number, and lesser number of tubules. These changes were more prominent in 30 day treatment groups characterized by hypertrophied seminal vesicles as compared to control group. Our result is in agreement with the work of Bahmanpour et al.[43] where it was shown that amitriptyline caused degenerative changes in testicular histo-architecture.

5. Conclusion

Finally, through this study, we can conclude that lambda cyhalothrin had induced changes in hematological, histopathological, biochemical, enzyme activities in the liver, kidney and testis of fresh water fish *C. batrachus*. These changes are duration dependent. The changes caused by lambda cyhalothrin by either affecting directly on the organ by modulating the enzymes as well as biochemical changes or through altering the hypothalamus hypophysial axis.

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