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ORIGINAL ARTICLE

# Effects of Cypermethrin on Serum Biochemistry and Liver Histopathology of *Anabas testudineus*

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**KEYWORDS**

Pesticide; Biochemical parameters; Histopathology; Liver

**ABSTRACT:** Cypermethrin is one of the most commonly used pesticides. In this study, the effects of cypermethrin on serum biochemistry and liver histology of *Anabas testudineus* were investigated. The fish have been exposed to sub-lethal concentrations of cypermethrin for the 7th, 14th, and 21st days and one control was considered. AST, ALT, and ALP showed concentration- and days-dependent increases in all experimental groups. Bilirubin levels increased significantly ($p<0.05$) in cypermethrin groups. No statistically significant difference in bilirubin levels was observed between the concentrations of 0.015 and 0.030 mg $L^{-1}$ on days 7th and 14th. Protein levels decreased in response to cypermethrin on all days when compared to controls. Statistically significant differences in protein levels weren't observed between all concentrations on days 7th and 14th and between concentrations of 0.015 and 0.030 mg $L^{-1}$ on days 21st. Light microscopy revealed hepatocyte hypertrophy, sinusoidal dilation, granular degeneration, congestion, pycnosis, and focal necrosis in the liver. AST ALT, ALP, bilirubin, protein levels, and histopathology can be used as possible markers for biological monitoring and chemical risk assessment in aquatic organisms.

## INTRODUCTION

Pesticides are chemicals commonly used in agricultural fields to control pests such as insects and weeds. Despite their beneficial role, uncontrolled and repeated applications of pesticides cause undesirable effects in non-targeted plants and animals. Pyrethroids, synthetic derivatives of pyrethrins, are insecticides derived from flowers of a chrysanthemum species (*Chrysanthemum cinerariaefolium*). Pyrethroids are very powerful neurotoxicants. Cypermethrin (CYP) is among the most commonly used synthetic pyrethroid [1]. CYP prolongs the opening of the sodium channels and causes hyperstimulation of the central nervous system in non-target organisms [2]. In addition to sodium channels, CYP can also affect chloride and calcium channels [3]. 96-hour LC50 values of CYP in fish species have been reported between 0.058 µg $L^{-1}$ and 0.4 mg $L^{-1}$. These values are very toxic to fish [4]. The 96-hour LC50 values of CYP for *Channa punctatus* were cited as 0.4 mg $L^{-1}$ [5], and 0.058 µg $L^{-1}$ for *Cyprinus carpio* [6]. Velmurugan et al. [7] suggested a 96-hour LC50 value of CYP for *Anabas testudineus* as 0.309 mg $L^{-1}$. The sensitivity of fish to pyrethroid pesticides may be due to the longer half-life of these substances in fish compared to birds and mammals [8].

Freshwater fish have been found useful as indicators of some pollutants due to their economic importance as a food source, their abundance in the freshwater ecosystem, their response to pollutants, and their easy adaptation to laboratory conditions. *A. testudineus* has an important place as a protein source in developing countries [9].

Serum biochemistry is an important physiological

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parameter of fish. Biochemistry parameters are used to observe the toxic effects of pollutants on fish [10]. AST (aspartate aminotransferase), ALT (alanine aminotransferase), ALP (alkaline phosphatase), bilirubin, and protein are liver function tests. ALT, AST, and ALP are liver-related enzymes. Serum bilirubin and protein serve to determine liver function. Of these tests, ALT and AST show hepatocellular damage, while ALP shows cholestasis. Bilirubin increase is involved in liver damage and cholestasis. The protein indicates liver synthesis capacity [11]. The liver is where serum proteins are synthesized. Serum proteins are an important measure for health status assessment.

Histopathological findings can provide insight into the body's health and its responses to stressors during exposure to pollutants, so they are widely used as biomarkers [12, 13]. If contaminants affect detoxification, histopathology is useful in examining the structure of the liver [14]. In fish, the liver is an important organ for the metabolism of toxic substances [15, 16]. The liver is an organ that is exposed to toxic substance due to its wide blood supply [17].

Several studies have previously been conducted on serum biochemical parameters [18, 19] or the histopathology of pyrethroid pesticides [20, 21] in various fish species. There are very few publications investigating hepatoxicity caused by pesticides in fish through both histopathology and serum biochemistry [22]. No such study of pyrethroid pesticides has been performed before. This research aimed to assess both serum biochemical parameters related to hepatic function and histopathology of hepatotoxicity caused by CYP in the *A. testudineus*.

## MATERIALS AND METHODS

*A. testudineus* (Anabantiformes: Anabantidae) specimens were collected from a spring in Red Hills Lake in Southern India. The fish were brought to the laboratory, and acclimatized to laboratory conditions for 20 days.

Fish were divided into four groups containing ten fish in each group and three repetitions were done for each group. Groups I, II, and III were exposed to 0.015, 0.030, and 0.045 mg $L^{-1}$ CYP, respectively. Group IV was used as a control and kept in water without CYP. In previous studies, it was shown that the 96-hour LC50 value of CYP for *A. Testudineus* was 0.309 mg $L^{-1}$ [7]. Selected concentrations were 5%, 10% and 15% of the 96-hour LC50 value of CYP. During the experiment and acclimatization, all fish were fed with commercial feed at 2% of their body weight two times a day.

Both fish exposed to CYP and control fish on days 7th, 14th, and 21st of the experimental study were anesthetized using 2-phenoxy ethanol. The blood drawn from the caudal vein of the anesthetized fish with the help of a sterile needle was collected in Eppendorf tubes without anticoagulants. Blood was centrifuged at 3,000xg for 10 minutes to obtain serum. The obtained serum was stored at -30°C until the biochemical analysis.

The AST and ALT levels were calculated using the UV kinetic process [23; 24]. The values for AST and ALT were expressed as units/L. ALP levels were calculated by the Tietz et al. [25] method. The values for ALP were expressed as units/L. Serum bilirubin levels were measured using a photometric bilirubin test updated by the Jendrassik and Grof methods [26]. The values for bilirubin were expressed in terms of mg $dL^{-1}$. Serum protein level was predicted by the Biuret method [27] and represented as g $dL^{-1}$.

The conformity of the parameter data with the assumption of the distribution of normality was assessed using the Kolmogorov-Smirnov method and its homogeneity with the Levene method. The two-way ANOVA was used to test measurements. In different comparisons, the corrected Bonferroni test was used.

Liver samples were taken from fish that were anesthetized. Bouin fluid was used to fix the liver samples. After dehydration, cleaning and paraffin embedding, sections were taken from paraffin blocks using a microtome. Sections were stained with Hematoxylin-Eosin. Changes were examined under a light microscope and photographed using a Leica photomicroscope.

## RESULTS

The blood serum biochemical levels were analyzed in control fish and exposed to CYP on the 7th, 14th, and 21st days and were presented in Table 1. The levels of serum AST, ALT, and ALP increased significantly in all groups exposed to CYP on the 7th, 14th, and 21st days. Days, concentrations, day-concentration interactions

when looking at the results for serum AST, ALT, and ALP levels showed statistically significant differences.

The levels of bilirubin increased significantly in CYP groups compared with the control group. No statistically significant difference in bilirubin levels was observed between the concentrations of 0.015 and 0.030 mg $L^{-1}$ on the 7th and 14th days. No statistically significant difference in bilirubin levels was observed between the 14th, and 21st days on the concentration of 0.015 mg $L^{-1}$.

Protein levels decreased in response to CYP exposures on the 7th, 14th, and 21st days when compared to controls. No statistically significant differences in protein levels were observed between all concentrations on days 7th and 14th, between concentrations of 0.015 and 0.030 mg $L^{-1}$ on day 21st and between 14th and 21st days on all concentrations.

**Table 1.** Serum biochemistry levels of fish exposed to cypermethrin.

| Biochemical parameters | Cypermethrin concentrations (mg $L^{-1}$) | 7th days | 14th days | 21st days |
|---|---|---|---|---|
| **AST (IU $L^{-1}$)** | Control | $97.00 \pm 5.12^{ax}$ | $96.00 \pm 6.04^{ax}$ | $99.00 \pm 5.98^{ax}$ |
| | 0.015 | $151.00 \pm 6.27^{bx}$ | $233.00 \pm 6.07^{by}$ | $400.00 \pm 6.65^{bz}$ |
| | 0.030 | $204.00 \pm 6.63^{cx}$ | $256.00 \pm 6.15^{cy}$ | $468.00 \pm 6.62^{cz}$ |
| | 0.045 | $240.00 \pm 6.31^{dx}$ | $272.00 \pm 17.26^{dy}$ | $525.00 \pm 6.22^{dz}$ |
| **ALT (IU $L^{-1}$)** | Control | $98.00 \pm 5.62^{ax}$ | $101.10 \pm 5.34^{ax}$ | $102.70 \pm 6.08^{ax}$ |
| | 0.015 | $117.00 \pm 6.16^{bx}$ | $132.00 \pm 6.73^{by}$ | $162.00 \pm 6.55^{bz}$ |
| | 0.030 | $131.00 \pm 6.78^{cx}$ | $159.00 \pm 6.96^{cy}$ | $174.00 \pm 6.52^{cz}$ |
| | 0.045 | $140.00 \pm 6.22^{dx}$ | $168.00 \pm 6.09^{dy}$ | $182.00 \pm 6.02^{dz}$ |
| **ALP (IU/L)** | Control | $89.55 \pm 3.12^{ax}$ | $91.02 \pm 5.03^{ax}$ | $90.07 \pm 5.42^{ax}$ |
| | 0.015 | $99.150 \pm 6.00^{bx}$ | $147.60 \pm 6.62^{by}$ | $214.00 \pm 6.11^{bz}$ |
| | 0.030 | $106.00 \pm 6.51^{cx}$ | $160.00 \pm 7.20^{cy}$ | $231.90 \pm 6.01^{cz}$ |
| | 0.045 | $129.00 \pm 5.79^{dx}$ | $179.00 \pm 6.01^{dy}$ | $252.00 \pm 11.30^{dz}$ |
| **BILIRUBIN (mg $dL^{-1}$)** | Control | $0.99 \pm 0.16^{ax}$ | $0.98 \pm 0.18^{ax}$ | $1.01 \pm 0.24^{ax}$ |
| | 0.015 | $1.29 \pm 0.34^{bx}$ | $1.60 \pm 0.29^{by}$ | $1.86 \pm 0.25^{by}$ |
| | 0.030 | $1.42 \pm 0.36^{bx}$ | $1.89 \pm 0.29^{by}$ | $2.80 \pm 0.17^{cz}$ |
| | 0.045 | $1.76 \pm 0.11^{cx}$ | $2.60 \pm 0.33^{cy}$ | $3.49 \pm 0.34^{dz}$ |
| **PROTEIN (g $dL^{-1}$)** | Control | $6.93 \pm 1.04^{ax}$ | $7.22 \pm 1.06^{ax}$ | $7.16 \pm 1.05^{ax}$ |
| | 0.015 | $4.70 \pm 0.67^{bx}$ | $3.27 \pm 0.82^{by}$ | $2.56 \pm 0.91^{by}$ |
| | 0.030 | $4.39 \pm 1.89^{bx}$ | $2.61 \pm 0.87^{by}$ | $2.32 \pm 0.53^{by}$ |
| | 0.045 | $4.250 \pm 1.25^{bx}$ | $2.30 \pm 0.57^{by}$ | $1.61 \pm 0.37^{cy}$ |

The values are mean ± SD (N = 9). a, b, c, and d indicate differences among concentrations for the same day. x, y, and z indicate differences among days for the same concentration. $p<0.05$.

Histopathology of the liver from control and experimental groups of fish is shown in Figures (1-4). The liver of the control fish showed normal structure. The basic structural element of the liver is the hepatocyte, also called the liver cell. These epithelial cells form the hepatic cell cords. There are sinusoids among the hepatic cell cords of the liver. Hepatocytes are polygonal cells with a nucleolus and a spherical nucleus (Figure 1).

On day 7th of exposure to 0.015 mg $L^{-1}$ CYP, the liver tissue of the fish showed hypertrophy of hepatocytes. In addition, dilatation of sinusoids and congestion were observed in the liver of fish exposed to 0.030 and 0.045 mg $L^{-1}$ CYP on day 7th (Figure 2).

Liver of fish exposed to 0.015 and 0.030 mg $L^{-1}$ CYP for 14 days showed hypertrophy of hepatocytes, granular degeneration, dilatation of sinusoids, and congestion (Figure 3). In addition, pycnotic nucleus and focal necrosis have been reported in liver of fish exposed to 0.045 mg $L^{-1}$ CYP on day 14th.

The liver of fish exposed to 0.015, 0.030, and 0.045 mg $L^{-1}$ CYP on day 21st showed hypertrophy of hepatocytes, granular degeneration, dilatation of sinusoids, congestion, pycnotic nucleus, and focal necrosis (Figure 4).

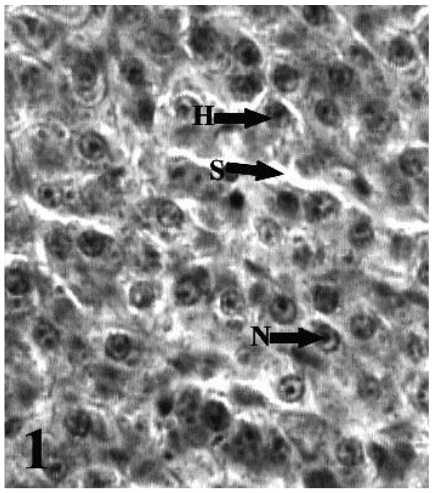


**Figure 1.** Photomicrographs of the liver of *Anabas testudineus*(Magnifications ×40).
(1) Control- (H) hepatocytes (S) sinusoids (N) nucleus.

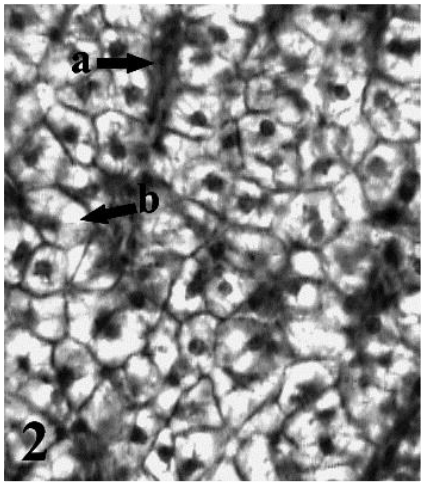


**Figure 2**.On day 7th of exposure to 0.045 mg $L^{-1}$ cypermethrin, the liver tissue of the fish
(a)dilatation of sinusoids and congestion, (b) hypertrophy of hepatocytes.

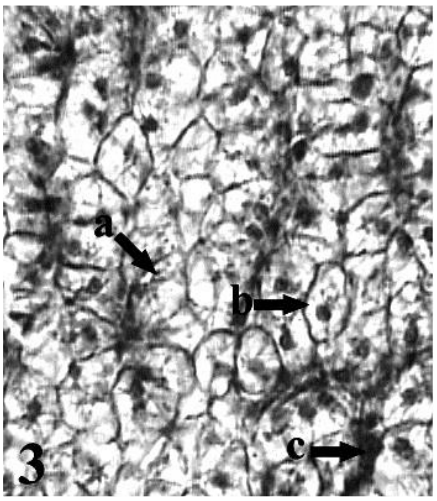


**Figure 3.**On day 14th of exposure to 0.030 mg $L^{-1}$ cypermethrin, the liver tissue of the fish
(a) granular degeneration, (b) hypertrophy of hepatocytes, (c) dilatation of sinusoids and congestion.

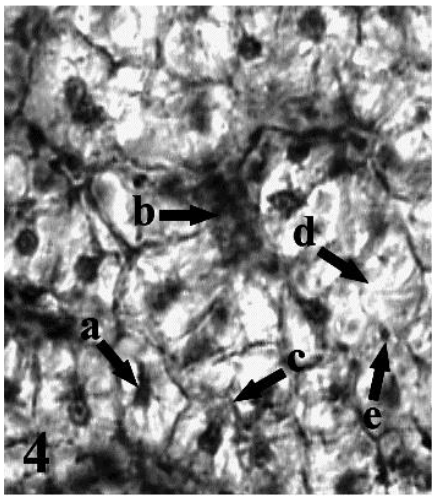


**Figure 4.** On day 21st of exposure to 0.045 mg $L^{-1}$ cypermethrin, the liver tissue of the fish (a) pycnotic nucleus, (b) dilatation of sinusoids and congestion, (c) hypertrophy of hepatocytes, (d) focal necrosis, (e) granular degeneration

## DISCUSSION

Biochemical parameters of blood provide important information in assessing the health status of the organism. These parameters are important for understanding the normal and pathological condition of fish and the toxicological effects in fish [28].

Enzymes used in liver damage assessment are ALT, AST, and ALP. AST and ALT are intracellular enzymes and are released into the blood due to hepatocyte damage. An increase in these enzymes in the blood suggests that the liver is damaged [29]. AST and ALT levels increased in all CYP exposures as compared with control values. These results are consistent with previous research results [30; 31]. The increases in AST and ALT levels obtained in this study serve as indicators of liver damage. Increased serum AST and ALT levels were observed in *Oreochromis niloticus* [18] and *Prochilodus lineatus* [32]. After exposure to CYP. It has been reported that deltamethrin caused increased ALT levels in *C. carpio* [33]. Velisek et al. [34] found increased AST levels in *Oncorhynchus mykiss* after exposure to CYP. Deltamethrin caused adverse effects resulting in a significant increase in AST and ALT activities [31]. Velisek et al. [35] found that AST and ALT levels in *O. mykiss* after acute exposure to bifenthrin were similar to

the control group. Increased levels of enzymes AST and ALT in response to λ-cyhalothrin were found [36].

ALP is an enzyme responsible for the detoxification, metabolism, and biosynthesis of macromolecules. An effect of this enzyme causes lesion formation in the tissues and dysfunction in the cells [31]. In this study, ALP levels increased in fish exposed to CYP. An increase in the levels of serum ALP of Nile tilapia, which was exposed to the subacute concentration of deltamethrin for 28 days was recorded [37]. CYP exposure caused increased serum ALP levels in *O. mykiss* [34]. The increased ALP level in serum is an indicator of liver injury.

Bilirubin is produced by the breakdown of heme and other porphyrin rings. Bilirubin, a bile pigment formed in the liver, increases in hemolytic diseases, hepatocellular diseases, or obstructed bile ducts [38]. In this study, serum bilirubin levels in the exposed *A. testudineus* showed an increasing trend when compared with the control fish. An elevated serum bilirubin level in *Sebastes schlegeli* exposed CYP was also recorded [30]. Increased serum bilirubin levels can be due to liver damage and bile duct obstruction.

In this study, protein levels decreased in response to all CYP exposures on the 7th, 14th, and 21st days when compared to controls. Proteins are the most important macromolecules in living things. These are important in physiological levels and metabolism in the cell [39]. The synthesis place of serum proteins is the liver. Serum proteins are important for health status assessment. Hypoproteinemia may be associated with pesticide exposure in fish. The serum protein level decreased in *Catla catla* after exposure to fenvalerate [40]. In a study that investigated the sublethal toxicity of cyhalothrin, it was found that serum protein level decreased in *C. carpio* when compared to the control [41]. CYP has been reported to cause hypoproteinemia in *Sebastes schlegeli* [30] and *C. carpio* [42]. Deltamethrin caused a decrease in serum protein levels in *O. niloticus* [31]. The decrease in serum protein content observed in fish after pesticide exposure indicates the physiological adaptation of the fish to pesticide stress. The high energy needed by animals to overcome this stress may have stimulated protein catabolism [43]. The decrease in serum proteins of fish exposed to pesticides can be attributed to decreased protein synthesis in the liver or protein loss due to necrosis.

Increased serum bilirubin levels and reduced serum protein content due to exposure to CYP in fish indicate an imbalance of biliary dysfunction in the fish liver during liver injury with toxic substances. In fish exposed to cypermethrin, blood parameters and hepatocyte damage worsened more than control normal depending on days and concentrations. Liver enzymes ALT, AST, and ALP are the most common tests used for investigating liver damage [11]. Histopathology is also used to explain liver damage [12; 13]. It can be known if the pesticide causes liver harm by looking at the amount of these enzymes in the blood. These enzymes are mainly present in the liver tissue in usually healthy individuals. A sign that liver cells have been killed is their release into the bloodstream. According to the results obtained from this experiment, ALT, AST, ALP values of fish exposed to CYP increased. The increase in the amount of these enzymes is proportional to the damage in liver histology. The rise in blood enzyme levels suggests an increase in damage to liver tissue. Increases in ALT, AST ALP, bilirubin, and decreases in protein indicate hepatocellular injury, according to the findings obtained from this experiment. Histopathological observations are correlated with the reported results of liver biochemical parameters.

In this study, CYP exposure in the liver of fish, granular degeneration, hepatocyte hypertrophy, sinusoid dilatation, congestion, pycnotic nucleus, and focal necrosis were observed. Similar results were also observed in the previous studies. Gulsoy [44] studied the histological sections of the liver in the *Xiphophorus helleri* after permethrin exposure and reported numerous changes such as hypertrophy and vacuole formation in hepatocytes, irregularity in hepatic cords, enlargement of sinusoids, atrophy, necrosis, and mononuclear leukocyte infiltration. Irregular nucleus shape, melanomacrophage aggregation, hepatolysis, and vacuole formation were reported in the liver of fish exposed to lambda-cyhalothrin [20]. Sharma and Jindal [21] studied the histological sections of the liver in the *C. catla* after CYP exposure and reported dilation of sinusoids, vacuolation, pycnosis, karyolysis, nuclear pleomorphism, and lymphocyte infiltration. These results indicated that

different pyrethroid pesticides and fish species showed similar histopathologies.

CYP caused major changes in the serum biochemistry and liver histology of exposed fish. As a result of the current research, it turned out that CYP is a powerful hepatotoxic pesticide against fish. AST ALT, ALP, bilirubin, and protein levels and histopathology can be used as possible markers for biological monitoring and chemical risk assessment in aquatic organisms.

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### *Conflict of interest*

The authors declared no conflict of interest.

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