Acta Biologica Turcica, 35(1), 5-13, 2022 Received 19 Jul. 2021; Accepted 02 Oct. 2021; Published Online 13 Jan. 2022

ACTA BIOLOGICA TURCICA

E-ISSN: 2458-7893, http://www.actabiologicaturcica.com

Research article

Toxic impact of chlorpyrifos, an organophosphate pesticide, on serum biochemical parameters of *Anabas testudineus*

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Abstract: The serum biochemical effects of *Anabas testudineus* were examined after exposure to sublethal concentrations of chlorpyrifos (0.125, 0.250 and 0.375 mg/L) for 7, 14 and 21 days. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) activities, bilirubin levels increased in response chlorpyrifos exposures at all exposure periods. On the 7th day, the sodium and potassium levels increased only in fish exposed to 0.375 mg/L of chlorpyrifos. On the 14th and 21st days, sodium levels increased depending on concentration, potassium levels were higher than the control. Calcium levels decreased on 14th and 21th days. Phosphorus levels decreased on 14th and 21 th days. On the 7th, 14th and 21st days, glucose levels increased and protein levels decreased. The cholesterol level was in a decreasing trend with increase in the concentrations of chlorpyrifos on 7th days. On the 14th and 21st days, cholesterol levels increased depending on concentration. This study shows that chlorpyrifos, in subletal concentrations, elicits serious metabolic disorders in *A. testudineus.*

Keywords: *Anabas testudineus*, Chlorpyrifos, Serum parameters, Enzyme activities, Metabolites, Ions

Citation: Velmurugan, B., Cengiz, E.I., Yolcu, M., & Uysal, E. (2022). Toxic impact of chlorpyrifos, an organophosphate pesticide, on serum biochemical parameters of *Anabas testudineus. Acta Biologica Turcica,* 35(1), 5-13.

Introduction

Pesticides are chemical compounds used to control various pests such as insects, rodents, fungi and unwanted plants in agriculture and to kill disease vectors like mosquitoes in public health. Pesticides not only affect pests but also potentially toxic to other organisms that including humans (Dikshith, 2013). Chlorpyrifos (O,O-diethyl-O-3,5,6-trichlor-2-pyridyl phosphorothioate) is a widely used organosphosphate insecticide in India (Majumder and Kaviraj, 2019). Chlorpyrifos reaches the surrounding waters by air entrainment or surface runoff, and accumulates in different aquatic organisms, especially fish, affecting them negatively. Chlorpyrifos inhibit the activity of acetylcholinesterase, the enzyme responsible for inactivating acetylcholine (ATSDR, 1997). Chlorpyrifos is very toxic for fish. Its 96 h LC50 value is found within the range of 0.5–15.395 µg/l (Huang and et al., 2020).

Biochemical parameters are used as potential biomarkers in various organisms because they are quite sensitive and show little variability between species (Agrahari et al., 2007). Blood biochemical parameters are a common diagnostic tool in fish toxicology and biomonitoring (Adams et al., 1996). The best physiological indicators of fish health are biochemical parameters.

Among aquatic species, fish have been the main targets of toxic contamination. Fish are useful

experimental models to evaluate the situation of water ecosystems exposed to environmental pollution (Das and Mukherjee, 2013). *Anabas testudineus* is important as a source of protein for people in developing countries (Zalina et al., 2011).

The present study aimed to evaluate the toxicological hazards of chlorpyrifos through assessment of its subletal exposure consequences, on the basis serum ALT, AST, ALP activities, bilirubin, glucose, protein, cholesterol, sodium, potassium and calcium levels on commercially important freshwater fish *A. testudineus*.

Materials and Methods

Fish and experimental design

Fish collected from a source in Red Hills Lake in India. Fish were acclimated to laboratory conditions for a period of 20 days. The photoperiod was set to be 12 hours light and dark. The temperature of water was adjusted to 27.50 ± 1.5 °C.

The fish were divided into four groups and placed in separate glass aquaria. Present study was performed in three replicates in order to ensure the reproducibility of the results. 10 fish were used for each group per replicate. Groups I, II and III were exposed to sublethal concentration of chlorpyrifos. Group IV was maintained in pesticide-free water to serve as control. The nominal concentrations of chlorpyrifos tested were 0.125, 0.250, 0.375 mg/L. These concentrations were chosen because they are lower than lethal concentrations for *A. testudineus.* In previous studies, the 96-hour LC50 value of chlorpyrifos for *A. testudineus* was found to be 2.5 mg/L (Velmurugan et al., 2015).

Throughout the experiments and acclimatization, control and experimental fish were fed once a day with commercial pellets at a rate of 2% of their body weight.

Blood sample collection

On the 7th, 14th and 21st days, both fish exposed to chlorpyrifos and control fish were anesthetized with 2-phenoxy ethanol. Blood samples were collected from anesthetized fish. Blood samples were collected in Eppendorf tubes without anticoagulants for serum collection. Serum was obtained by centrifugation of blood at 3.000 rpm for 10 min. Serum samples were stored at -30°C until the analysis biochemical parameters.

Biochemical assay

The activities of AST and ALT were determined using UV kinetic method (Bergmeryer et al., 1986a, b). The enzyme activities were estimated indirectly by the rate of NADH oxidation at 340 nm. The AST and ALT values were expressed as units/L.

The activity of ALP was estimated by a kinetic UV method (based on liberation of p-nitrophenol p-nitrophenyl phosphate). ALP activity was measured by the method of Tietz et al. (1983). The ALP values were expressed as units/L.

The levels of serum bilirubin were estimated by using photometric test for bilirubin method modified by Jendrassik and Grof (1938). The bilirubin values were expressed as mg/dL.

Sodium and potassium levels were analysed by using Flame photometer (Hald and Burkett Mason, 1958). The sodium and potassium values were expressed as mg/dL.

The levels of serum calcium were estimated by using photometric test for calcium according to CPC method (Gitelman, 1967). The calcium values were expressed as mg/dL.

The levels of serum calcium were estimated by photometric test for phosphorus according to the method developed by Daly and Ertingshausen (1972). The phosphorous values were expressed as mg/dL.

The level of serum glucose was estimated using enzymatic colorimetric test by GOD-POD method (Barham and Trinder, 1972). The glucose values were expressed as mg/dL.

The level of the serum protein was estimated by the Biuret method (Gornall et al., 1949). The protein values were expressed as g/dL.

The levels of serum cholesterol were estimated by enzymatic photometric test using CHOD-PAP method (Deeg and Ziegenhorn, 1983). The cholesterol values were expressed as mg/dL.

Statistical analysis

One way ANOVA was used to evaluate measurements in this study. The Tukey HSD, Dunnet test was used for multiple comparisons. Normal distributions were evaluated using the Kolmogorov-Smirnow test and homogeneity was evaluated using Levene's test. All data were analyzed using the statistical package SPSS version 15.0 for Windows. The significance of test results was ascertained at $p<0.05$.

Table 1. Serum biochemistry levels of *Anabas testudineus* exposed to sublethal concentrations of chlorpyrifos.

Parameters	Chlorpyrifos concentrations (mg/L)	7 days	14 days	21 days
AST (units/L)	Control	98.00±5.70ax	95.00±6.53ax	113.00±6.85ay
	0.125	112.70±6.85bx	219.00±6.36by	396.00±6.38bz
	0.250	186.00±6.36cx	237.00±7.63cy	442.00±6.57cz
	0.375	229.00±6.06dx	280.00±7.20dy	490.00±6.75dz
ALT (units/L)	Control	97.00±6.48ax	110.10±6.01ay	103.70±6.95axy
	0.125	100.00±6.15ax	130.40±6.67by	156.00±6.02bz
	0.250	110.00±6.67bx	135.00±6.25by	162.00±5.89bcz
	0.375	128.00±6.55cx	145.00±5.96cy	169.00±6.58cz
ALP (unitsU/L)	Control	87.55±3.98ax	92.00±5.86axy	95.00±5.25ay
	0.125	96.00±4.03bx	116.00±5.98by	220.40±6.85bz
	0.250	126.00±5.72cx	166.00±6.67cy	260.00±6.68cz
	0.375	142.00±5.58dx	207.00±6.63dy	310.00±6.48dz
BILIRUBIN (mg/dL)	Control	0.99±0.16ax	0.96±0.19ax	1.23±0.42ax
	0.125	1.24±0.25bx	1.67±0.18by	2.19±0.42bz
	0.250	1.44±0.21bx	2.58±0.17cy	3.08±0.32cz
	0.375	1.75±0.13cx	3.84±0.17dy	4.74±0.29dz
SODIUM (mg/dL)	Control	128.10±6.62ax	126.00±6.31ax	132.00±6.41ax
	0.125	124.00±6.52ax	137.00±6.31by	148.00±6.53bz
	0.250	127.40±5.50ax	146.00±6.09cy	157.00±7.04cz
	0.375	136.40±6.80bx	151.00±7.27dcy	165.00±6.02dz
POTASSIUM (mg/dL)	Control	4.69±1.68ax	5.02±1.32ax	5.07±1.29ax
	0.125	3.04±0.96ax	11.24±3.13by	9.71±2.19by
	0.250	3.31±1.36ax	11.98±3.17by	11.31±2.29by
	0.375	7.24±1.58bx	12.40±5.15by	12.360±2.67by
CALCIUM (mg/dL)	Control	24.76±4.59ax	27.43±6.73ax	26.670±7.66ax
	0.125	22.00±4.66ax	9.52±2.14by	5.12±1.27bz
	0.250	21.49±3.67ax	8.35±2.38by	4.74±1.21bz
	0.375	20.66±5.25ax	7.68±2.35by	4.53±1.18by
PHOSPHORUS (mg/dL)	Control	13.59±3.37ax	14.29±2.89ax	14.70±3.26ax
	0.125	12.60±4.43ax	12.87±3.46abx	11.30±2.54bx
	0.250	11.99±3.05ax	9.74±1.75bcxy	8.98±2.68bcy
	0.375	10.58±1.86ax	6.84±2.06cy	6.34±2.17cy
GLUCOSE (mg/dL)	Control	36.40±5.44ax	37.40±5.84ax	38.30±5.78ax
	0.125	47.40±5.54bx	61.00±6.63by	71.40±6.28bz
	0.250	50.30±6.08bx	65.30±6.87by	86.30±6.22cz
	0.375	62.40±6.54cx	74.00±6.77cy	108.00±7.35dz
PROTEIN (g/dL)	Control	6.93±1.31ax	7.24±1.05ax	7.14±1.07ax
	0.125	5.09±1.31bx	3.83±0.95by	3.11±0.83by
	0.250	4.45±1.59bx	3.34±1.10bxy	2.43±0.60bcy
	0.375	3.93±1.28bx	2.88±0.92bxy	2.03±0.57cy
CHOLESTROL (mg/dL)	Control	315.90±5.45ax	309.10±5.88ay	322.20±4.69az
	0.125	293.60±8.06bx	323.10±4.95by	422.70±4.72bz
	0.250	273.40±7.01cx	356.10±5.22cy	492.40±4.72cz
	0.375	232.50±5.80dx	384.20±5.10dy	568.90±5.51dz

Values are expressed as mean ± SD (N = 10), Letters "a," "b," "c"and "d"and indicate differences between groups at the same time, and letters "x", "y" and "z"and indicate differences between times for the same group. $p<0.05$.

Results

The serum biochemical parameters were analysed in *A. testudineus* exposed to sublethal concentrations of chlorpyrifos on 7th, 14th and 21th days. Results of serum biochemical parameters of the control and chlorpyrifos-exposed *A. testudineus* are given in Table 1.

Serum AST and ALP activities of *A. testudineus* increased in response to chlorpyrifos exposure when compared to control on the 7th, 14th and 21st days. The serum ALT activities increased also on 7th and 14th days. Changes in the serum AST, ALT and ALP activities in the *A. testudineus* are shown in the Figures 1-3.

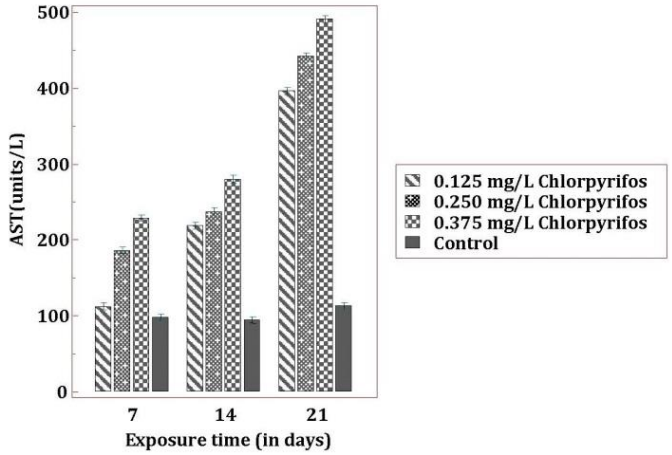

Figure 1. AST levels in *Anabas testudineus* exposed to sublethal concentrations of chlorpyrifos for 7, 14 and 21 days. Data are expressed as mean± SD (N = 10). $p<0.05$.

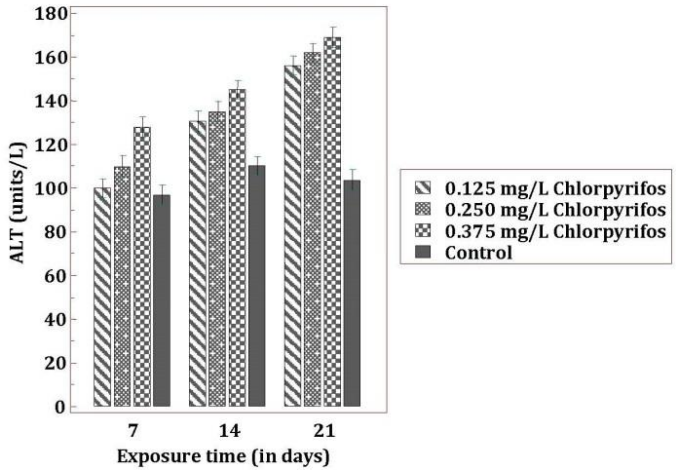

Figure 2. ALT levels in *Anabas testudineus* exposed to sublethal concentrations of chlorpyrifos for 7, 14 and 21 days. Data are expressed as mean± SD (N = 10). $p<0.05$.

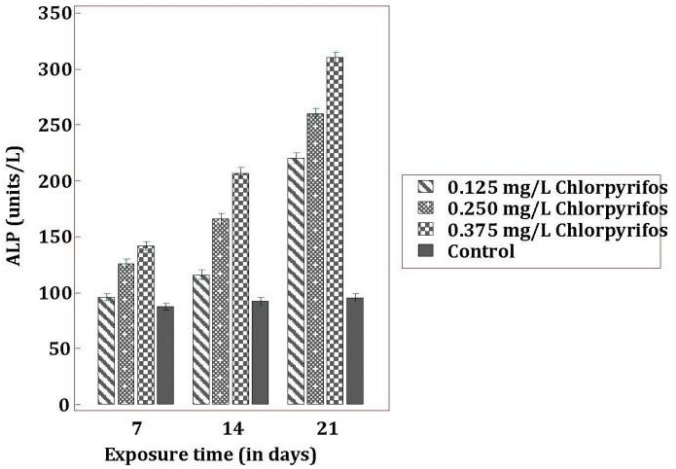

Figure 3. Serum ALP levels in *Anabas testudineus* exposed to sublethal concentrations of chlorpyrifos for 7, 14 and 21 days. Data are expressed as mean± SD (N = 10). $p<0.05$.

Serum bilirubin levels in the fish exposed to sublethal concentrations of chlorpyrifos showed an increasing trend when compared with the control fish (Figure 4). An overall elevation was observed throughout the exposure period. The maximum increase was found at concentration of 0.375 mg/L on 21 days of exposure.

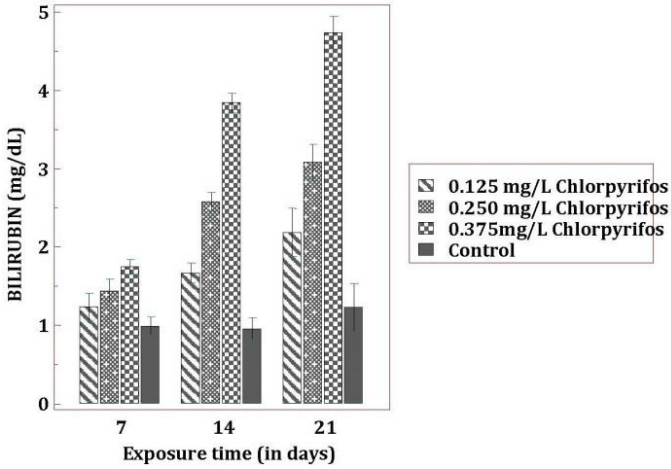

Figure 4. Serum bilirubin levels in *Anabas testudineus* exposed to sublethal concentrations of chlorpyrifos for 7, 14 and 21 days. Data are expressed as mean± SD (N = 10). $p<0.05$.

On the 7th day, the sodium and potassium levels increased only at the concentration of 0.375 mg/L. On the 14th and 21st days, sodium levels increased depending on concentration and the potassium levels were higher than the control. Significant changes in sodium and potassium levels are shown Figures 5 and 6.

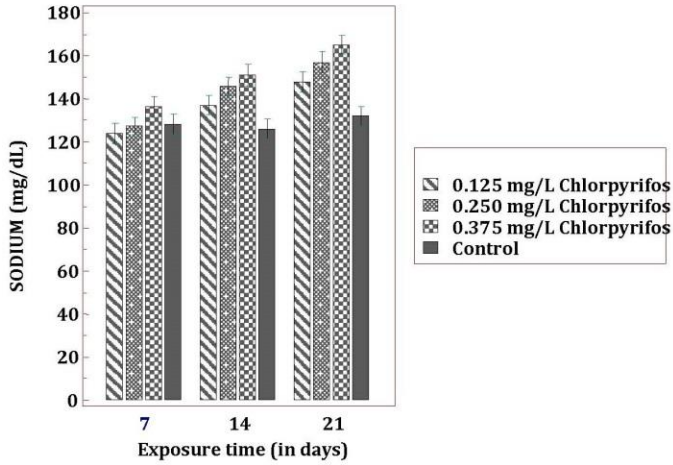

Figure 5. Serum sodium levels in *Anabas testudineus* exposed to sublethal concentrations of chlorpyrifos for 7, 14 and 21 days. Data are expressed as mean± SD (N = 10). $p<0.05$.

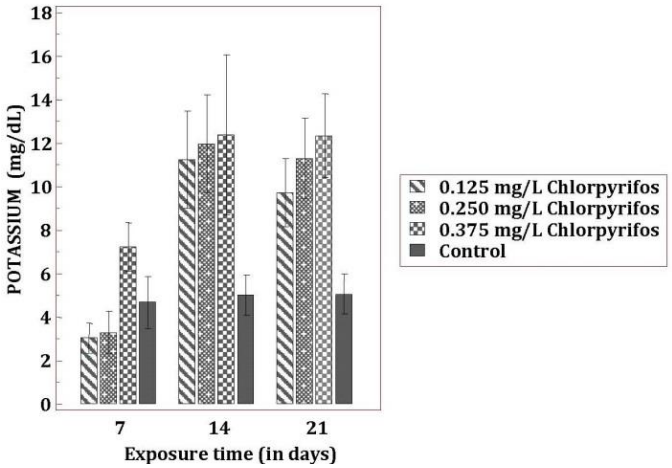

Figure 6. Serum potassium levels in *Anabas testudineus* exposed to sublethal concentrations of chlorpyrifos for 7, 14 and 21 days. Data are expressed as mean± SD (N = 10). $p<0.05$.

Calcium and phosphorus levels decreased on 14th and 21th days. The maximum decrease was found on the 21st day at concentration of 0.375 mg/L (Figures 7 and 8).

Glucose levels increased on the 7th, 14th and 21st days. The maximum increase was found at concentration of 0.375 mg/L on 21 th days of exposure (Figure 9).

Protein levels decreased on the 7th, 14th and 21st days. The reductions in protein levels are presented in Figure 10.

The cholesterol level was in a decreasing trend with increase in the concentrations of chlorpyrifos on 7th days. On the 14th and 21st days, cholesterol levels increased depending on concentration (Figure 11).

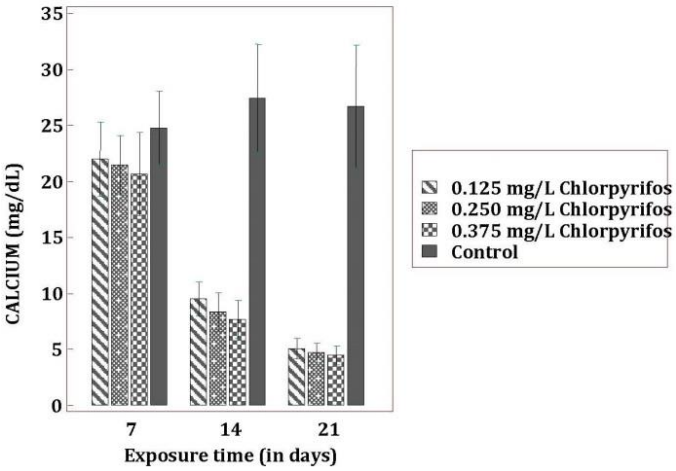

Figure 7. Serum calcium levels in *Anabas testudineus* exposed to sublethal concentrations of chlorpyrifos for 7, 14 and 21 days. Data are expressed as mean± SD (N = 10). $p<0.05$.

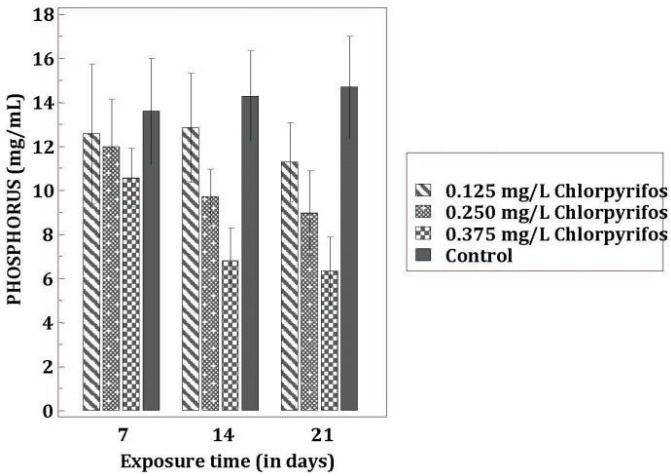

Figure 8. Serum phosphorus levels in *Anabas testudineus* exposed to sublethal concentrations of chlorpyrifos for 7, 14 and 21 days. Data are expressed as mean± SD (N = 10). $p<0.05$.

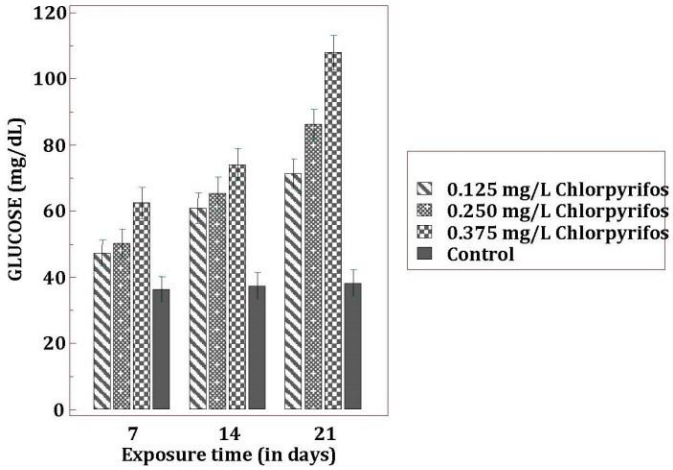

Figure 9. Serum glucose levels in *Anabas testudineus* exposed to sublethal concentrations of chlorpyrifos for 7, 14 and 21 days. Data are expressed as mean± SD (N = 10). $p<0.05$.

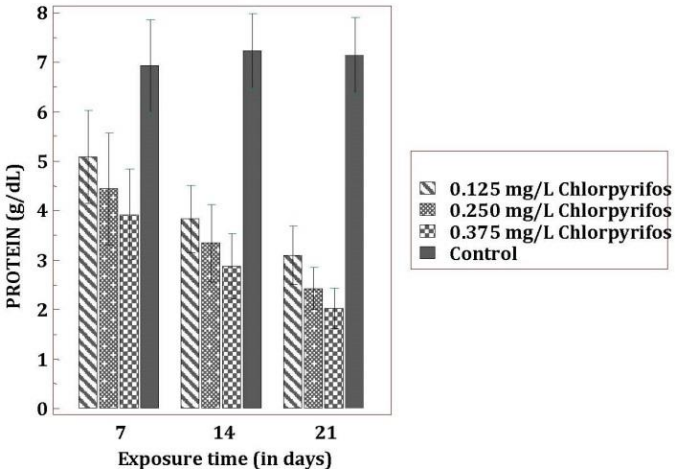

Figure 10. Serum protein levels in *Anabas testudineus* exposed to sublethal concentrations of chlorpyrifos for 7, 14 and 21 days. Data are expressed as mean± SD (N = 10). $p<0.05$.

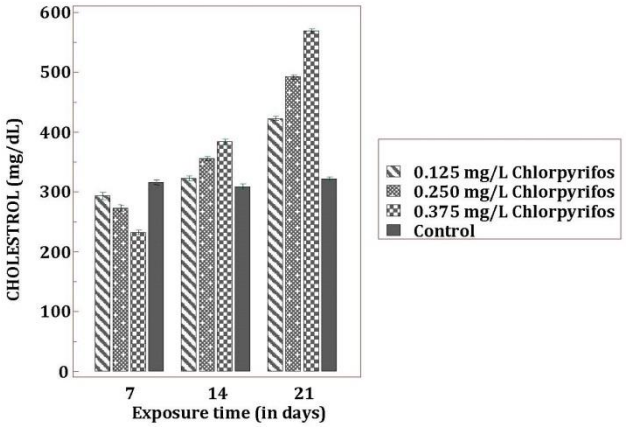

Figure 11. Serum cholestrol levels in *Anabas testudineus* exposed to sublethal concentrations of chlorpyrifos for 7, 14 and 21 days. Data are expressed as mean± SD (N = 10). $p<0.05$.

Discussion

Biochemical profiles of blood can provide important information about the internal environment of the organism. The evaluation of biochemical characteristics in fish has become an important means of understanding normal and pathological processes and toxicological impacts (Borges et al., 2007; Sudova et al., 2009).

Transaminases are enzymes that play an important role in protein and amino acid metabolism. The activity of AST and ALP increased in all experimental groups as compared to the control values. The serum ALT levels increased also on 7th and 14th days. These results are in accordance with the results of previous investigators on freshwater fish (Rao, 2006; Hatami et al., 2019). The significant increases in serum AST, ALT and ALP activities were demonstrated in *Oreochromis mossambicus* (Rao, 2006) and *Cyprinus carpio* (Hatami et al., 2019) exposed to monocrotophos and chlorpyrifos, respectively. Serum AST and ALT activities increased in *Oncorhynchus mykiss* (Banaee et al., 2011), *Cirrhinus mrigala* (Haider and Rauf, 2014), *Clarias gariepinus* (Al-Otaibi et al., 2019), after exposure to diazinon.

The liver is the center of detoxification of chemicals. One of the indicators of liver damage is changes in transaminase activities (Asztalos et al., 1988).

Bilirubin is a bile pigment formed in the liver. Bilirubin consists of the breakdown of heme and other porphyrin rings. Serum bilirubin level in fish exposed to chlorpyrifos showed an increasing trend when compared with the control fish. The increased levels of serum bilirubin were also recorded in *Heteropneustes fossilis* exposed to Nuvan (2, 2 dichlorovinyl dimethylphosphate) (Shaikh and Gautam, 2014). However, the decreased serum bilirubin levels were recorded in *Clarias albopunctatus* exposed to roundup (Okonkwo et al., 2013). The change in the bilirubin was not observed in *Clarias gariepinus* after paraquat dichloride toxicity (Ogamba et al., 2011). The increased levels of serum bilirubin may be attributed to liver damage and obstruction of the bile duct.

Electrolits play a vital role in several body functions. The monovalent ions sodium and potassium are involved in neuromuscular excitability, acid base balance and osmotic pressure, whereas divalent cation calcium facilitate neuromuscular excitability, enzymatic reactions and retention of membrane permeability. Further, inorganic phosphate acts as a major cytoplasmic buffer and is the basis of energy exchange (Guyton, 1991). In fish exposed to chlorpyrifos, serum sodium and potassium levels increased; calcium and phosphorus levels decreased. The significant increases in serum Na and K levels were reported in *Cirrhinus mrigala* exposed to malathion, dimethoate and chlorpyrifos (Rani et al., (2017). Malathion, dimethoate and chlorpyrifos caused a decrease in serum calcium levels in *Cirrhinus mrigala* (Rani et al*.,* 2017). The acute effect of diazinon in *Cyprinus carpio* caused significantly higher sodium and potassium concentrations and significantly lower concentrations of calcium and phosphorus, than the compared with the control group (Luskova et al., 2002). Changes in electrolyte levels may be explained as a result of the effects of pollutants on organs involved in osmoregulation, on endocrine system, on metabolism, or on active transport processes.

Increase of blood glucose level is a common sign of stress. Glucose increase is a general response of fish to acute and sublethal pollutant effects (Luskova et al., 2002). The previous studies, serum glucose levels have been reported to increase during organophosphate pesticide exposure (De Aguiar et al., 2004; John, 2007; Islam et al., 2019). The increases were reported in the glucose level in the blood of *Brycon cephalus* (De Aguiar et al., 2004), *Mystus vittatus* (John, 2007), *Pangasianodon hypophthalmus* (Islam et al., 2019) exposed to monocrotophos, metasystox and sumithion, respectively. The significant increases in serum glucose level were demonstrated in *Clarias batrachus* (Narra, 2017) exposed to dimethoate; and *Cyprinus carpio* (Luskova et al., 2002), *Cirrhinus mrigala* (Haider and Rauf, 2014) and *Clarias gariepinus* (Al-Otaibi et al., 2019) exposed to diazinon. Disorders cause the high

levels of blood glucose in carbohydrate metabolism appearing in the condition of pesticide stresses.

Proteins are the most important and abundant macromolecules in living beings, which play a vital role in the architecture and physiology of the cell and in cellular metabolism (Mommsen and Walsh, 1992). Several other investigators have also reported the depletion of the serum protein in fish exposed to organophosphate pesticides (Luskova et al., 2002; Ramesh and Saravanan, 2008; Shaikh and Gautam, 2014). Nuvan (2, 2 dichlorovinyl dimethylphosphate) caused a decrease in serum protein level in *Heteropneustes fossilis* (Shaikh and Gautam, 2014). A significant decrease in serum protein levels was demonstrated in *Cyprinus carpio* exposed to diazinon (Luskova et al., 2002) and chlorpyrifos (Ramesh and Saravanan, 2008). The decrease in protein content of fish exposed to pesticides also indicated the physiological adaptability of the fish to compensate for pesticide stress. To overcome the stress, the animals require high energy. This energy demand might have led to the stimulation of protein catabolism (Sancho et al., 1997).

In this study, the cholesterol levels of fish exposed to chlorpyrifos initially decreased and then increased. Hatami et al. (2019) reported decreasing levels of serum cholesterol in *Cyprinus carpio* exposed to chlorpyrifos. In contrast, increased cholesterol levels of *Leiarius marmoratus* and *Pseudoplatystoma reticulatum* exposed to glyphosate have been reported by de Moura et al. (2017). Hatami et al. (2019) explained that the decrease in serum cholesterol levels was caused by lower absorption in the intestine, biosynthesis disorders in the liver and malnutrition. An increase in serum cholesterol is indicative of liver dysfunction (John, 2007). The liver is the crucial organ in the synthesis and excretion of cholesterol. Therefore, any type of obstruction in the liver, either intra or extrahepatic, will cause an increase in total cholesterol levels of the serum (Okechukwa and Auta, 2007). An increase in the bloods cholesterol content suggested that chlorpyrifos either enhanced the cholesterol production or inhibited excreation through the bile duct. It may also be due to the necrosis of liver cells.

Conclusion

This study shows that chlorpyrifos, in sublethal concentrations, reveals serious metabolic disorders in *A.testudineus*. The changes in serum parameters may be the result of pesticide damage to organs such as liver, gill and kidney. Consequently, the examined parameters can be used as good biomarkers of organophosphate pollution.

Conflicts of Interest

No potential conflict of interest was reported by the authors.

Ethical approval

All applicable national guidelines for the care and use of animals were followed.

Funding

This study was supported by the Jawaharlal Nehru Memorial Fund (JNMF) Scholarships for PhD Programme in India.

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