International Journal of Educational Research and Development
www.multidisciplinaryarticle.net
Online ISSN: 2664-7095, Print ISSN: 2664-7087
Received: 14-04-2024, Accepted: 06-05-2024, Published: 30-05-2024
Volume 6, Issue 2, 2024, Page No. 102-105

Endosulfan's impact on the liver of *Clarias gariepinus* (B): Biochemical analysis

P Verma[1*], S Kurikose[2], D B Sawarkar[1]
[1] Department of Zoology, Hislop College, Nagpur, Maharashtra, India
[2] Department of Zoology, N.H. College, Bramhapuri, Maharashtra, India
Corresponding Author: payalrverma@gmail.com

Abstract
The most common toxicants in aquatic environments are organochlorides. Endosulfan is an insecticide containing organochlorines that is neurotoxic and damages DNA strands. The liver is essential to the metabolic changes that contaminants undergo during the detoxification process. The liver is responsible for the metabolic changes that toxins undergo during the detoxification process. The liver performs several vital bodily processes, such as regulating metabolism, synthesizing plasma proteins, storing energy, storing certain vitamins and trace minerals, transforming and excreting steroids, and detoxifying xenobiotics. However, the body reacts negatively to higher amounts of harmful substances. Biochemical parameters provide a rapid and reliable means of monitoring a pesticide's effects on aquatic biota and, ultimately, the environment. The quantity of biochemical components in various organs reveals how harmful a pesticide is. The liver tissue of freshwater catfish *Clarias gariepinus* exposed to sublethal amounts of endosulfan was evaluated in the current study for total protein and total carbohydrate content. The current study's findings indicate that endosulfan treatment of *Clarias gariepinus* liver tissues gradually reduced their protein and carbohydrate level. This reduction is directly proportional to time of exposure and concentration of the insecticide.

Keywords: Biochemistry, *Clarias gariepinus,* endosulfan, liver, total carbohydrate, total protein

Introduction
Organochlorides are the most prevalent toxicants found in aquatic environments. Since the 1940s, these chlorinated hydrocarbons have been extensively used in agriculture and mosquito control. Endosulfan is an organochlorine insecticide that is neurotoxic and breaks DNA strands. It also interferes with the cell's damage response mechanism, which makes DNA strand repair more difficult. Endosulfan increases agricultural productivity by reducing damage to crops. However, it may also be dangerous to non-target organisms like fish since it alters their physiology, metabolism, behaviour, and reproduction. This could ultimately damage the population's ability to survive (Altinok & Capkin, 2007) [1]. The metabolic alterations that pollutants go through during the detoxification process depend on the liver. Toxin exposure and accumulation frequently results in liver lesions and other histological changes (Reddy & Rawat, 2013 [14], Verma *et al.*,2022a [29], Kuriakose *et al.*, 2022b). Salamat and Zarie (2012) [17] state that the liver is responsible for many essential biological functions, including energy storage, metabolic regulation, the synthesis of plasma proteins, the transformation and excretion of steroids, and the detoxification of xenobiotics. Fish liver is in direct contact with toxic compounds that the body absorbs from contaminated water since it is the primary organ of metabolism and detoxification. The health condition of the fish exposed to a particular toxic chemical will be reflected in its liver glycogen content. Carbohydrate is stored mainly in liver in the form of glycogen. Changes in glycogen content of these tissues are clear evidence of unfavourable conditions of fish. The lipid concentration in the body and the relative contribution of lipid to energy production in fish varies with environmental condition, stage of maturity, nutritional state and with different parts of the body. Myers *et al.*, (1987) [9] typically divided fish hepatic alterations into various groups and assessed each category according to its relative importance as a measure of toxicant exposure. The gastrointestinal tract is often in close touch with compounds that are hazardous to humans, making it extremely sensitive to these substances. Research on the intestines provides important insights on the toxicity of these substances.

Material and methods
Young *Clarias gariepinus* fish (12-13 gm and 10-11 cm long) were purchased from the market and acclimatized under laboratory conditions for 15 days and later treated with Endosulfan 35% EC (Endocel).
Chronic Toxicity measures long-term effects of exposure (typically 21-28 days). Sub lethal or safe level concentrations were derived from 96h LC 50 (APHA, 1992) [2]. In the present study the 96 h LC_{50} value of Endosulfan in *Clarias gariepinus,* was found to be 4.355µg/l with a 95% confidence limit ranging from 3.428µg/l (lower confidence limit) to 5.651µg/l (upper confidence limit). LC_{50} values of 24, 48 and 72 h of Endosulfan in *Clarias gariepinus* are 5.912µg/l, 5.459µg/l, 4.927µg/l respectively (Verma *et al.*,2022 [29]; Kuriakose *et al.*, 2024).
Total proteins were measured with Bradford Reagent (Bradford, 1976) and total carbohydrates analysis was done by the method of Dubois *et al.* (1956) [4], (Verma *et al.*,2022 [29]; Kuriakose *et al.*, 2024).

Observation
In the present study it is estimated the total protein and total carbohydrate in liver tissue of fresh water cat fish *Clarias gariepinus* exposed to sub lethal concentrations of Endosulfan for 5, 10 and 15 days was evaluated (Table 1.).

Table 1: Total protein content (µg/g) of liver tissues of *Clarias gariepinus* exposed to sublethal concentrations of Endosulfan.

	Control	0.215 µg/l	0.430 µg/l	0.645 µg/l	0.860 µg/l
5 days	31.52 ± 0.32	29.78 ±0.71	25.86 ± 1.86	14.42 ± 0.96	9.66 ± 0.02
10 days	32.52 ± 0.32	24.76 ± 0.82	17.49 ± 0.29	9.06 ± 0.86	4.25 ± 0.13
15 days	31.22 ± 2.32	21.62 ± 1.20	14.73 ± 0.03	7.32 ± 0.17	4.19 ± 0.58

Results of the present study show that the total protein content of liver tissue decreased with the increase of concentration of Endosulfan. Liver tissue treated at 0.215 µg/l (lowest sub lethal concentration) resulted in protein content 29.78 ±0.71 µg/g, 24.76 ± 0.82 µg/g and 21.62 ± 1.20 µg/g, for 5, 10 and 15 days, respectively. The protein content showed a gradual decrease with increase in concentration and at 0.860 µg/l (highest sublethal concentration) it is exhibited lowest protein contents 9.66 ± 0.02µg/g (5 days), 4.25 ± 0.13 µg/g (10 days) and 4.19 ± 0.58 µg/g (15 days).

Total protein content decreased with the increase in the number of days of exposure also. On the 5 days, the values are 29.78 ±0.71µg/g at 0.215 µg/l, 25.86 ± 1.86µg/g at 0.430 µg/l, 14.42 ± 0.96µg/g at 0.645 µg/l, 9.66 ± 0.02 µg/g at 0.860 µg/l. By the 15 days the values decreased to 21.62 ± 1.20 µg/g at 0.215 µg/l, 15.73 ± 0.03µg/g at 0.430 µg/l, 8.32 ± 0.17µg/g at 0.645 µg/l, 4.19 ± 0.58 µg/g at 0.860 µg/l, respectively.

Liver tissue exposed to the sub lethal concentrations of Endosulfan showed a significant decrease in total carbohydrate content compared to control values for 5 days, 10 days and 15 days exposure. Total carbohydrate content decreased with the increase of concentration and increase in the number of days of exposure (Table 2).

Table 2: Total carbohydrate content (µg/g) of liver tissues of *Clarias gariepinus* exposed to sub lethal concentrations of Endosulfan.

	Control	0.215 µg/l	0.430 µg/l	0.645 µg/l	0.860 µg/l
5 days	12.280 ± 1.650	7.32 ± 1.560	4.35 ± 2.65	3.86 ± 0.82	2.52 ± 0.83
10 days	13.240 ± 1.670	6.56 ± 0.310	4.45 ± 0.92	2.52 ± 0.26	2.48 ± 0.02
15 days	11.560 ± 1.030	5.96 ± 0.360	4.95 ± 0.86	2.17 ± 0.76	2.12 ± 1.26

The carbohydrate content of liver tissue in fishes treated with Endosulfan showed a decrease, proportional to the increase of concentration. Liver tissue exposed to the lowest sub lethal concentration (0.215 µg/l) for 5 days, 10 days and 15 days showed high carbohydrate contents 07.32 ± 1.560 µg/g, 6.56 ± 0.310 µg/g and 5.96 ± 0.360 µg/g, respectively. It decreased gradually with the increase in concentration of pesticide and decreased carbohydrate contents of 2.52 ± 0.83µg/g (5 days), 2.48 ± 0.02µg/g (10 days) and 2.12 ± 1.26µg/g (15 days) were observed at 0.860 µg/l (Highest sub lethal concentration).

Results of the present study showed that a decrease in carbohydrate content of liver tissue, proportional to the increase of exposure period of Endosulfan was observed. Exposure of tissues to the lowest concentration (0.215 µg/l) for 5 days shows the value as 7.32 ± 1.560 µg/g. It decreased to 5.96 ± 0.360µg/g (15 days). Exposure of tissues to the highest concentration (0.860 µg/l for 5 days shows the value as 2.52 ± 0.83 µg/g. It decreased to 2.12 ± 1.26µg/g on the 15 days.

Discussion

The findings of the current research exhibit a gradual decline in protein levels in liver tissue of *Clarias gariepinus* exposed to Endosulfan and Imidacloprid. The protein content peaked at the 5-day mark but reached its lowest point after 15 days of treatment. Interestingly, the lowest concentration of pesticides resulted in the highest protein content, while the highest concentration led to the lowest. This decrease in total protein was directly proportional to both the duration of exposure and the concentration of the pesticide. Since fish have minimal carbohydrate reserves, protein becomes a primary alternative energy source to fulfil heightened energy requirements. The ability of animals to withstand stress hinges significantly on their capacity for protein synthesis. The decline in protein content suggests increased proteolytic activity, potentially utilizing protein products for metabolic purposes and causing tissue damage. Prior studies by Rao *et al.* (1987) [13], Palanichamy *et al.* (1989) [11], and Sheela *et al.* (1992) [19] also reported a declining trend in tissue protein levels. Other research, such as that by Tilak *et al.* (2001) [25], observed total protein depletion in liver tissues of *Labeo rohita* exposed to Chlorpyrifos. Significant decreases in liver protein content due to exposure to Endosulfan were noted in *Clarias batrachus* by Tripathi & Verma (2004a) [27], and to Fenvalerate by Tripathi & Verma (2004b) [28]. Sobha *et al.* (2007) [20] documented liver protein reduction in *Catla catla* exposed to Cadmium Chloride, while Tilak *et al.* (2009) [26] observed decreases in liver protein in *Channa punctatus* exposed to Alachlor. Ganeshwade (2011) [5] found depleted protein content in the liver tissue of the *Puntius ticto* exposed to Dimethoate, suggesting a direct relationship between toxicity, concentration, and exposure time. This decline in protein content may be attributed to structural changes in the liver, inhibition of protein synthesis, or an increase in degradation rate to amino acids, possibly to fuel the TCA cycle and meet heightened energy demands during stress. Overall, the decrease in tissue protein content may signify a reallocation of energy to cope with stress-induced demands or altered enzyme activities.

Rohankar *et al.* (2012) [16] observed a noteworthy decline in both soluble and insoluble proteins in the liver tissues of freshwater fish *Channa punctatus* following exposure to Phosphamidon for 24, 48, and 72 hours. Tataji & Kumar (2016) [24] reported protein depletion in the liver tissues of *Channa punctata* exposed to Butachlor and Machete. Borale & Khodake (2017) [3] documented a decrease in liver protein levels in *Mystus bleekeri* exposed to Sodium Arsenite. Ogundiran & Fawole (2018) [10] found reduced protein levels in *Clarias buthupogon* and *Heterobranchus longifilis* from polluted water, attributing the reduction to impaired protein synthesis due to liver cirrhosis induced by toxicants or pollutants. Protein metabolism plays a crucial role in the

compensatory mechanism during stressful conditions, as highlighted by Mastan & Rammayya (2010) [8]. Sobha *et al.* (2007) [20] observed a decline in liver glycogen levels in *Catla catla* exposed to Cadmium Chloride.

Carbohydrates stored in the liver are typically the first nutrients utilized in response to stress. Susan *et al.* (2010) [23] reported a significant decrease in glycogen content in liver tissues of *Labeo rohita* and *Cirrhinus mrigala* exposed to sublethal and lethal concentrations of technical grade Fenvalerate. Ganeshwade (2011) [5] noted a decrease in liver glycogen content in *Puntius ticto* exposed to Dimethoate. Stalin & Das (2012) [21] observed a significant reduction in glycogen content in *Cirrhina mrigala* exposed to Fenthion. Suneetha (2012) [22] observed depletion of total glycogen in liver tissues of *Labeo rohita* exposed to Endosulfan and Fenvalerate. Satyavardhan (2013) [18] reported glycogen depletion in liver tissues of *Ctenopharyngodon idella* exposed to Fenvalerate and Malathion. Reddy *et al.* (2015) [15] noticed decreased total glycogen in the liver of *Labeo rohita* exposed to Confidor. Patnaik *et al.* (2016) [12] reported a decrease in glycogen in the liver tissue of *Anabas testudineus* after exposure to Naphthalene.

In line with the findings of the current study, *Clarias gariepinus* treated with Endosulfan exhibited a decreasing trend in carbohydrate content in liver and intestine tissues compared to control fishes. Additionally, as the concentration increased, carbohydrate content decreased, with the highest carbohydrate content found in the lowest concentration and the lowest content in the highest concentration of Endosulfan. Carbohydrate content also decreased with an increase in the number of days of exposure.

References

1. Altinok I, Capkin E. Histopathology of rainbow trout exposed to sublethal concentrations of Methiocarb or Endosulfan. Toxicol Pathol,2007:35:405-410.
2. APHA. Standard methods for the examination of water and wastewater,18th ed. Washington D.C.: APHA, 1992.
3. Borale RP, Khodake SP. Sodium arsenite induced histochemical changes in the liver of fish Mystus bleekeri. IOSR J Eng,2017:7(12):5-9.
4. Dubois M, Gilles KA, Hamilton JK, Roberts PA, Smith F. Calorimetric method for determination of sugars and related substances. Anal Chem,1956:28(3):350-356.
5. Ganeshwade RM. Biochemical changes induced by Dimethoate in the liver of freshwater fish Puntius ticto (Ham.). Biol Forum Int J,2011:3(2):65-68.
6. Kurikose S, Verma P, Sawarkar DB. Histopathological effect of the insecticide imidacloprid on the liver of Clarias gariepinus (Burchell, 1822) (Siluriformes: Clariidae). Int J Fish Aquat Res,2022:7(2):52-56.
7. Kurikose S, Verma P, Sawarkar DB. Biochemical effects of endosulfan on Clarias gariepinus (Burchell, 1822) kidneys. Int J Multidiscip Educ Res,2024:9(2):6-8.
8. Mastan SA, Rammayya PJ. Biochemical profile of Channa gachua (Ham) exposed to sublethal doses of Dichlorovas (DDVP). Internet J Toxicol,2010:8:27-32.
9. Myers MS, Rhodes LD, McCain BB. Pathologic anatomy and patterns of occurrence of hepatic neoplasms, putative preneoplastic lesions, and other idiopathic hepatic conditions in English sole (Parophrys vetulus) from Puget Sound, Washington. J Natl Cancer Inst,1987:78(2):333-363.
10. Ogundiran, Fawole,2018.
11. Palanichamy S, Arunachalam A, Bhaskaran P. Effect of pesticide on protein metabolism in the freshwater catfish Mystus vittatus. J Ecobiol,1989:1:90-97.
12. Patnaik L, Raut D, Panda D, Nayak S. Naphthalene induced biochemical changes in Anabas testudineus. J Biodivers Environ Sci,2016:8(2):154-158.
13. Rao SK, Sreenivasa MK, Reddy B, Swamy KS, Sreeramulu C. Effect of Benthicarb on protein metabolism of fresh water teleost fish Sarotherodon mossambicus. Indian J Environ Health,1987:29:45-15.
14. Reddy PB, Rawat SS. Assessment of aquatic pollution using histopathology in fish as a protocol. Int Res J Environ Sci,2013:2(8):79-82.
15. Reddy A, Veeraiah K, Rao T, Vivek C. Studies on some biochemical changes in the tissues of the Fresh water fish Labeo rohita (Hamilton) exposed to Confidor. J Int Acad Res Multidiscip,2015:3(1):323-329.
16. Rohankar P, Zade V, Dabhadkar D, Labhsetwar N. Evaluation of impact of Phosphamidon on protein status of Freshwater Fish Channa punctatus. Indian J Sci Res,2012:3(1):123-126.
17. Salamat N, Zarie M. Using of fish pathological alterations to assess aquatic pollution: A review. World J Fish Marine Sci,2012:4(3):223-231.
18. Satyavardhan K. Effect of Fenvalarate TM and MalathionTM on biochemical constituents of freshwater fish, Ctenopharyngodon idella (Valenciennes). World Appl Sci J,2013:27(5):649-655.
19. Sheela M, Mathivanan R, Muniandy S. Impact of Fenvaerate on biochemical studies of different tissues in the fish Channa striatus (Bloch). Environ Ecol,1992:10:547-549.
20. Sobha K, Poornima A, Harini P, Veeraiah K. A study on biochemical changes in the fresh water fish, Catla catla (Hamilton) exposed to the heavy metal toxicant Cadmium Chloride. Kathmandu Univ J Sci Eng Technol,2007:1(4):1-11.
21. Stalin IS, Das SMS. Biochemical changes in certain tissues of Cirrhina mrigala (Hamilton) (Cyprinidae: Cyprniformes) exposed to Fenthion. Int J Environ Sci,2012:2(3):1268-1277.
22. Suneetha K. Effects of Endosulfan and Fenvalerate on carbohydrate metabolism of the freshwater fish, Labeo rohita (Hamilton). Int J Pharm Pharm Sci,2012:4(1):262-268.
23. Susan AT, Sobha K, Veeraiah K, Tilak KS. Studies on biochemical changes in the tissues of Labeo rohita and Cirrhinus mrigala exposed to Fenvalerate technical grade. J Toxicol Environ Health Sci,2010:2(5):53-62.
24. Tataji PB, Kumar MV. Biochemical changes induced by Butachlor and Machete 50% EC to the freshwater fish Channa punctata (Bloch). Int J Sci Res,2016:5(3):2048-2052.
25. Tilak KS, Veeraiah K, Ramana Kumari GV. Toxicity and effect of Chlorpyrifos to the freshwater fish Labeo rohita (Hamilton). Pollut Res,2001:20:443-445.
26. Tilak KS, Wilson RP, Butchiram MS. Effects of alachlor on biochemical parameters of the fresh water fish, Channa punctatus (Bloch). J Environ Biol,2009:30(3):420-426.

27. Tripathi G, Verma P. Endosulfan-mediated biochemical changes in the freshwater fish Clarias batrachus. Biomed Environ Sci,2004a:17(1):47-56.
28. Tripathi G, Verma P. Fenvalerate-induced changes in a catfish, Clarias batrachus: metabolic enzymes, RNA and protein. Comp Biochem Physiol C Toxicol Pharmacol,2004b:138(1):75-79.
29. Verma P, Kurikose S, Sawarkar DB. Histopathological effect of endosulfan on the liver of Clarias gariepinus (Burchell, 1822) (Siluriformes: Clariidae). J Int Acad Res Multidiscip,2022:10(2):1-10.