The Bioscan 10(4): 1539-1542, 2015
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

BIOCHEMICAL CHANGES IN THE PROFILE OF CARBOHYDRATE, PROTEIN AND TRIGLYCERIDES IN THE BLOOD OF *CLARIAS BATRACHUS* DUE TOSUBLETHAL TREATMENT WITH INSECTICIDE ROGOR.

NANDANI SHARAN, RITA GUPTA AND PRAKRITI VERMA
Department of Zoology, B. S. College, MagadhUniversity, Bodh Gaya - 800 012
Department of Zoology, Patna University , Patna, Bihar - 800 005
email: ritaguptapatna@yahoo.com

KEYWORDS
Clarias batrachus
Carbohydrates
Protein
Rogor
Triglycerides

Received on :
27.07.2015

Accepted on :
20.11.2015

***Corresponding author**

ABSTRACT

In present study, the toxicity of insecticide rogor on biochemical parameters (carbohydrate,protein and triglycerides) of fresh water fish *Clariasbatrachus* were estimated.The LC_{50}(96 hours) of rogor for fish have been calculated as 4.5μl/L . Two sub lethal concentration selected in the experimental protocol were 1μl/L and 2.5μl/L of Rogor and accordingly stock solutions of rogor were prepared using distilled water.Fishes were sacrificed after 5th,10th,15thdays of exposure.The total protein count shows great variations ,at 1μl/L , the total protein first decreased on 5thday by 39.7% but increased on 10thand 15thday although it was 15.8% lower than the protein count of controlled fish. However, on 2.5μl/L the serum protein of treated fish increased by 10.5% on 5thday of the treatment and on 15thit showed an increase of 39.5% as compared to the controlled fish. The glucose level also increased in comparison to the controlled fish. On 1μl/L , the serum glucose increased by 15% on 5thday of the treatment but on 10th and 15thday it decreased by 3.9% and 15% respectively. Whereas on 2.5μl/L ,the serum glucose increased by 18% on 5thday,33% on 10thday and 29% on 15thday of the treatment. Triglycerides shows reduction in both doses , at 1μl/L triglyceride reduced by 1.7%, 16.7% and 19.5% respectively, on 5thday ,10thday and 15thday . On 2.5μl/L the decline in the triglyceride of treated fishes in comparison to control fish, on 5th, 10thand 15thday are 30.6%, 39.6% and 41.6% respectively. This shows that rogor exposure hampers the nutritional value of these commercially important fishes

INTRODUCTION

Urbanization, advanced agricultural operations and rapid industrial growth have encouraged more and more usage of pesticide.It has been established that a substantial portion of the pesticide utilized in agriculture and forestry also finds its way by seepage along with leaching out of the pesticides residue containing soil pollutants, polluting the aquatic environment, endangering the aquatic population thriving therein, particularly fishes. Pesticide poisoning in fishes is considered to be very serious as fish form a major food resource for mankind. Both human being andfishes life is at stake.

Rogor is dominant pesticide widely used by agriculturists in Bihar. Pesticide when leeched out in water enters into fish body by passive diffusion through gills, epithelial tissues or intestinal tract. Biotransformation of pesticides in fish appears to be carried out largely in liver followed by kidney, plasma, intestine microflora, brain and gonads (Pesonen *et al*., 1985). Rogor is an organophosphate with acetylcholine esterase inhibiting ability (Dutta *et al*., 1995). It bears acute and chronic toxicity and posses reproductive, tetratogenic, mutagenic and carcinogenic effects (Hayes, 1982 ; Hellenbeck, 1985). Effect of sublethal concentration of lead nitrate on the blood profile of Clariasbatrachus was studied by Nehar *et al*. (2010), showed various changes in the blood parameters The acid phosphatase, alkaline phosphatase and C-reactive protein levels raised in the experimental fish.Endosulfan induced histopathologicalalteration in the liver, kidney and gills of L.rohita was studied by Inderabai *et al*. (2010), observed pathological lesions leading to necrosis of hepatocytes, glomorulus of kidney and primary and secondary gill filaments of gills, which was on par with the duration of exposure of fish to endosulfan and thereby affect homeostasis of fish, L. rohita.

The effect of rogor on biochemical profile of fish have been worked by Gupta and Srivastava (1984) ;Haider and Inbaraj (1986); Gill *et al*. (1991); Ghosh and Chatterjee (1985); Choudhary and Nath (1985). In the present investigation rogor induced alteration in the biochemical profile of carbohydrate, protein and triglycerides present in the blood of *Clariasbatrachus* have been done.

MATERIALS AND METHODS

Live and healthy species of *Clariasbatrachus* of average weight 60 $\pm$ 10 gm and 4.5" $\pm$ 2" length were procured from various wet lands of Dighadiyara, Patna. The fishes were brought to the laboratory, disinfected with 0.1 % $KMNO_4$ solution and kept for acclimatization in the standard laboratory condition . To maintain normal water temperature, cooler and exhaust were used around aquarium .The aerated tap water was

changed daily. After 48 hours, fishes were fed with pellet of wheat flour and eggs @ 5 % of their body weight. After, two weeks of properly acclimatization fishes were grouped into sets of six each.

In experimental protocol, commercially brand "Rogor (EC 30 %)" has been purchased from the local supplier. The 96 hours LC_{50} of rogor was calculated by APHA (2000) for *Clariasbatrachus* was calculated as 4.5 μl/L. The two doses considered in the experimental protocol was 1μl/L and 2.5μl/L of Rogor and accordingly stock solution of rogor were prepared using distilled water. Fishes were then treated with 1μl/L (lower) and 2.5 μl/L (upper sublethal) for 15 days. The solution was changed regularly. Fishes were sacrificed after 5th, 10th, and 15th day of exposure. After schedule period of exposure, the serum sample from controlled and treated groups were collected and assessed for different biochemical analysis specially serum total protein content, total glucose and triglycerides.

Blood sample were collected by puncture in heart ,left for half an hour and clear serum was decanted off from the supernatant ,centrifuged at 3000 r.p.m. for 15 minutes for three successive intervals. clear supernatant serum was decanted in a clean dry vial and stored in deep fridge at 20ºC for biochemical analysis.Blood serum analysis was done on BT260 Plus-semi-Automatic Analyzer

The serum glucose was measured by using the enzymatic GOD-POD method, End Point. (Juaristi *et al.,* 1995) following oxidation of glucose by glucose oxidase (GOD) to produce gluconate and hydrogen peroxide, which is then coupled with 4 amino-antipyrene and phenol in the presence of peroxidase (POD) to yield a red quinoeimine dye that is measured at 505 nm. The observation at 505nm is proportional to the concentration of glucose in the sample.

The total protein was measured by using Biuret method which is based on the reaction between the peptide bonds of protein and cu^{2+},that produces a blue-violet colored complex in alkaline solution (Hartree,1972, Lowry, *et al.*, 1951).

Total triglycerides content was estimated by enzymatic GPO/PAP method .Serum triglycerides are hydrolyzed to glycerol and free fatty acids by lipoprotein lipase , glycerol is converted to glycerol-3-phosphate,which is then oxidized by glycerol phosphate oxidase(GPO) to yield hydrogen peroxide. The oxidative condensation of 4-Chlorophenol and 4-aminophenazone in the presence of peroxidase and hydrogen peroxide produces a rose coloured dye which is measured at 550nm (Trinder, 1970; Young, 2000)

RESULTS AND DISCUSSION

Variation in biochemical profile of blood serum, that is total protein count, total glucose count and triglyceride content of both control and treated group of fishes have been shown in Table 1 and 2.

Total serum protein shows a great alteration. On lower sublethal dose i.e.1μl/L the serum protein shows decline by 39.7% on 5th day of exposure but on prolonged exposure the protein count increased significantly ($p<0.05$) ,although it was 15.8% lower than the protein content of controlled fish. However, on higher sublethal dose of pesticide i.e. 2.5μl/L the serum protein of treated fish increased significantly ($p<0.05$) by 10.5% on 5thday of the treatment and on 15thit showed significant($p<0.05$) increase of 39.5% as compared to the controlled fish as depicted in the text graph-1. Increase in protein content may be due to increase in protein synthesis due to increased protein synthesizing enzymes secretion under stress .Similar is the finding of Rajeshwar Rao *et al*. (1983) who suggested that incline in protein content might be due to pesticidal stress and the energy was derived from carbohydrate metabolism. Present result is in agreement with the result of Kabeer (1979) who observed increased in protein content in fish, *Tilapia mossambica* treated with methyl parathion and malathion. Shivaprasad Rao *et al*. (1980) reported incline in protein level due to increased level of protease and synthetic potentiality of proteins in various tissues under pesticidal impact. Magar R.S. and Afsar Shaikh (2012) investigated increase in muscle and stomach protein content of *Channa punctatus* under exposure to sublethal concentration of malathion. Ganeshwade *et al*. (2012) observed that on the acute exposure of rogor a decrease in the level of protein in testis, ovary, brain, muscles, liver, and gills was seen whereas increased protein level was observed in kidney.

However, most of the workers found decline in protein content in various tissues of fish under different stress conditions. Yaganabano *et al*. (1981) and Bano (1982) showed decrease

Table 1: Showing alteration in biochemical composition of *C.batrachus* on exposure to 1μl/L of Rogor for fifteen consecutive days

Sl. No.	Parameter	Period of exposure of Rogor on experimented fishes in days			
		control	5th days	10th days	15th days
1	Total protein (g/dl)	3.831 ± 0.110	2.335 ± 0.017*	2.817 ± 0.024*	3.223 ± 0.109*
2	Total glucose(mg/dl)	98.78 ± 2.247	100.496 ± 4.429	94.88 ± 1.25*	83.82 ± 0.459*
3	Triglycerides (mg/dl)	600.2 ± 1.128	540.5 ± 1.023*	500.1 ± 1.147*	482.7 ± 0.795*

Values are expressed in Mean ± SD of six replicates in each case.*$P<0.05$, significant when student's test was applied between control and experimental mean.

Table 2: Showing alteration in biochemical composition of *C.batrachus* on exposure to 2.5μl/L of Rogor for fifteen consecutive days

Sl. No.	Parameter	Period of exposure of Rogor on experimented fishes in days			
		control	5th days	10th days	15th days
1	Total Protein (g/dl)	3.831 ± 0.110	4.206 ± 0.001*	2.628 ± 0.016*	5.304 ± 0.100*
2	Total glucose (mg/dl)	98.78 ± 2.247	117.38 ± 1.11*	132.1 ± 19.008*	128.10 ± 1.200*
3	Triglycerides (mg/dl)	600.2 ± 1.128	416.2 ± 1.584*	362.4 ± 1.270*	350.3 ± 2.023*

Values are expressed in Mean ± SD of six replicates in each case;*$p<0.05$, significant when student's test was applied between control and experimental mean

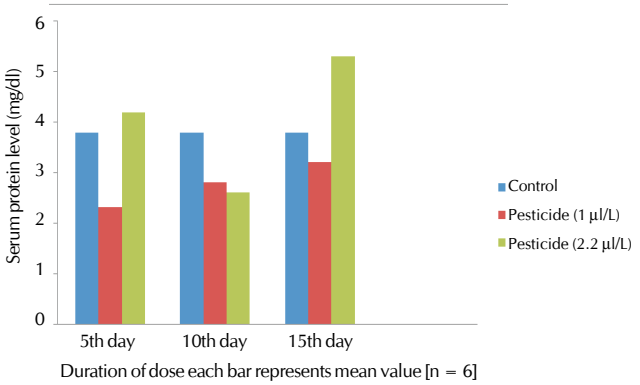

Figure 1: Fluctuation of protein level in Rogar treated *Clarias batrachus* in comparison to control

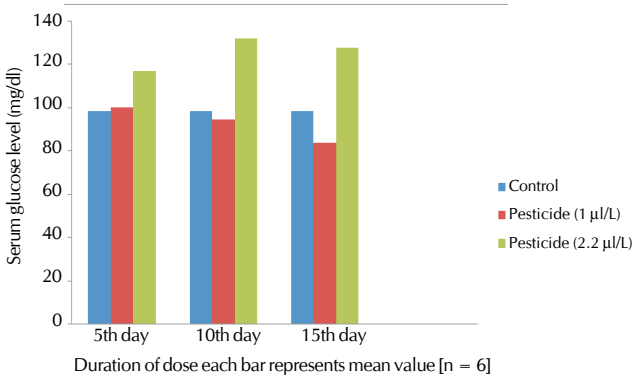

Figure 2: Fluctuation of glucose in Rogar treated *Clarias batrachus* in comparison to control

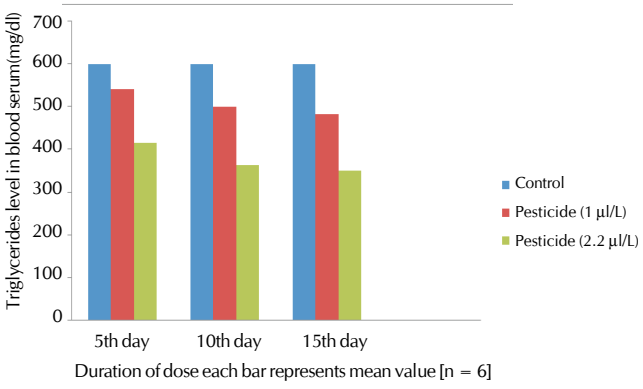

Figure 3: Fluctuation in triglycerides level in the boodof Rogar treated Clarias batrachus in comparison to control

in the total protein content of selected tissue and cholesterol content of serum and liver in *Clarias batrachus* exposed to eldrine. Venkataramana *et al*. (2006) showed impact of malathion on cardiac muscle of gobind fish, *Glosso gobiusgiuris* (Ham). They concluded along with glycogen, cholesterol and protein showed significant decrease at higher concentration of malathion.

Total serum glucose shows great variations. The text graph 2 shows, on lower sublethal dose, the serum glucose increased by 15% on 5th day of the treatment but on 10th and 15th day it decreased by 3.9% and 15% respectively. Whereas on higher sublethal dose, the serum glucose increased by 18% on 5th day, 33% on 10th day and 29% on 15th day of the treatment. The result is significant ($p<0.05$) under both the sublethal doses of rogor treatment except on fifth day under 1μl/L of rogor dose. So there is overall increase in serum glucose of treated fishes as compared to the controlled fish, shows great stress level under the impact of pesticide. The present finding is in agreement with the finding of Alpana Arya (2014) who also observed an increase in glucose and SGPT level in the serum of *Clarias batrachus* when exposed to mercury in compare to the control value. Health (1995) reported that the effects are cortisol mediated that is released from adrenal cortex, that in turn helps in glycogen storage in liver by reducing the use of glucose by tissues and it keeps blood glucose at higher concentration. Similar increase in serum glucose level was reported in *A.anguilla* exposed to femitrothion and endosulfan (Gimeno *et al 1994.*)and in *Clarias batrachus* exposed to malathion (Mukhopadary *et al*. 1980) as well as in *C. gariepinus* treated with λ-cyhalothrin (Ogueji *et al*. 2007).

Although, most of the workers found decline in serum glucose level when treated with rogor. Begum and Vijavaraghavan (1999) also reported reduced muscle glycogen in *C. batrachus* treated with the pesticide rogor. Similarly, Ndubuisi *et al*. (2014) found the liver glycogen and serum glucose concentrations decreased significantly ($p<0.05$) with increasing λ-cyhalothrin test concentration and duration of exposure. The decrease in the serum carbohydrate content is due to its mobilization from muscle to coelomic fluids as consequence of toxicant induced hypotoxic and anoxic condition and direct utilization for energy generation. (Reddy *et al.*,1984).

The serum triglyceride of treated fishes show decline, in both lower and higher sublethal doses of rogor, in comparison to the controlled fish. On lower sublethal dose, on 5th day,10th day and 15th day the triglyceride reduced by 1.7%, 16.7% and 19.5% respectively. On higher sublethal doses, the decline in the triglyceride of treated fishes in comparison to control fish, on 5th, 10th and 15th day are 30.6%, 39.6% and 41.6% respectively as shown in text graph 3. The result is significant ($p<0.05$) under both the sublethal doses of rogor. Decrease in lipid content may be due to energy synthesis by triglyceride oxidation under stressed condition. Similarly, significant decrease in triglycerides content in blood plasma of *C. batrachus* by mercury has been reported by Alpana Arya(2014). B. Lal and T. P Singh (1987), also reported a decrease in triglyceride due to sublethal concentration of malathion in *C. batrachus*.

Sublethal concentrations of rogor can alter the blood parameters which may cause histopathological alterations in tissues of fish exposed to these for a long period of time. However, it is important that the residual effects of these pesticides in different body tissues of fish are detrimental and inimical to mankind as they are ultimately consumed by the humans.

REFERENCES

APHA. 2000. Standard methods of examination of water and waste water 21sted. APHA,AWWA and WPCF *Amarican Public Health association,Washington DC.USA.*

Arya, A. 2014. Evaluation of biochemical and histochemical changes following the combined treatment of mercury and cadmium in a fresh water cat fish, *C.batrachus*. *International J. Pharmacy and pharmaceutical Sci.* **6:** 10.

Begum, G. and Vijayaraghavan, S. 1999. Effect of acuteexposure of the organophosphate insecticiderogor on some biochemical aspect of *C.batrachus* (Linn). *Environ. Res. (Series A).* **80:** 80-84.

Chaudhary, H. S. and Nath, K. 1985. Nickel induced hyperglycemia in the fresh water *Colisa fasceatus* water, air and soil pollution. **24:** 173-176.

Bano, Y. 1982. Effect of aldrin on serum and liver constituents of fresh water cat fish, *Clarias batrachus*. Proc. *Indian. Acad. Sci*. **19:** 27-32.

Dutta, H. M., Munshi, J. S. D., Dutta, G. R., Singh, N. K. Adhikari, S. and Richmonds, C. R. 1995. Age related differences in the inhibition of brain acetylcholinesterase activity of *H.fossilis* (Bloch) by malathion Comp. *Biocam. Physiol. IIIa. No. 2.* pp. 331-334.

Ganeshwade, R. M., Rokade, P. B., Sonwane, S. R. 2012. Impact of dimethoate on protein content in the fresh water fish *Puntius ticto.The Bioscan* **7(1):** 153-155.

Gosh, T. K. and Chatterjee, S. K.1985. Effect of chromium on tissue energy reserve in a fresh water fish. *Sarotherodon mossambicus*. *Environ. Ecolol.* **3:**178-179.

Gill,T. S., Pande, J. and Tiwari, H. 1991. Effects of endosulfan on blood and organ chemistry of fresh water fish, *Barbus conchonius*. *Ecotoxicol. Environ. Saf.* **21:** 80-91.

Gimeno, L., Ferrando, M. D., Samehez, S. and Andreu, E. 1994 Endolulfan effect on liver and blood of the eel *A. anguilla*. Comp. *Biochem. Physiol.* **108:** 343-348.

Gupta, A. B. and Srivastava, A. R. 1984. Phenol induced changes in the carbohydrate metabolism in the Indian catfish, *H. fossilis*. *Environ. Biol. Fishes*.**10:** 221-224.

Haider, S. and Inbaraj, R. M. 1986. Relative toxicity of technical material and commercial formulation of malation and endosulfan to a fresh water fish,*Channa punctatus. Ecotoxicol. Environ. Saf.***11:** 347-351.

Hallenbeck, W. H. and Cunninghams, K. M. 1985. *Pescitides and human health, New york, Springes-Verlag.* pp. 97-104.

Hartree, E. F. 1972. Analytical Biochemistry.48,422-427.

Hayes,W. J. 1982. Pesticide studied in man Batlimore, M. D Williams and Wilkins. pp. 205-215.

Indirabai, W. P. S, Tharani, G. G. and Seetha, P. 2010. Impact of sublethal concentration of endosulfan on biochemicals and histology of organ tissues of fresh water fish *Labeo rohita.The Bioscan.* **5(2):** 215-218.

Juaristi, E. and Gabriel, C. 1995. The anomeric effect. CRC Press,10 ISBN 0849389410 http:// wapedia.mobi/en/Glucose?t=9. p. 9.

Kbeer, A. S. I. 1979. Studies on some aspects of protein metabolism and associated enzymes system in fresh water teleost.*Tilapia mossambica* subjected to malation exposure, *Ph. D. Thesis submitted to S. V. University,Tirupati, India.*

Lal, B. and Singh, T. P. 1987. Changes in tissue lipid levels in the freshwater catfish, Clariasbatrachus associated with reproductive cycle. *Fish Physiol. Biochem.* **3:** 191-201.

Lowry, O. H., Rosenberg, N. J., Farr, A. L. R. and all, R. J. 1951. *J. Biological Chemistry*. **164:** 321-329.

Nehar, S., Kumar, C., Mishra, K. and Kumari, M. 2010. Lead induced alteration in the blood profile of air breathing catfish *Clarias batrachus*. *The Bioscan.* **(2):** 335-341

Magar, R. S. and Shaikh, A. 2012. Biochemical changes in protein and amino acids in *C.punctatus* in response to sublethal treatment with the insecticide malathion. *DAMA. Int. vol.1, no.3.*

Mukhopadary, P. K. and Dehadrai, P. V. 1980. Biochemicalchanges in the air breathing catfish *C.batrachus* exposed to malathion. *Environ. Pollout. A.* **22:** 144-158.

Ogueji, E. O. and Auta, J. 2007. The effect of sublethal dosesof lambda-cyhalothrin on some biochemicalcharacteristics of the African catfish *C. gariepinus. J. Biol. Sc.* **7:** 1473-1477

Oluah, Ndubuisi Stanley and Chineke, A. C. 2014. Alteration in the biochemical parametersof African cat fish*C.gariepinus* exposed to sublethal concentration of lambda-cyhalothrin. *Annals of Environmental Sci.* **8:** 1-7.

Pesonen, M., Anderson, T. and Fortum, L. 1985. Characterization and induction of cytochrome P450 in the rainbow trout. *Mar. Environ. Res.***17:** 106-108.

Rajeshwar, Rao, M., Sivaprasad Rao, K., Srinivasulu Reddy, M., Sambasiva Rao, K. R. S. and Reddy, N. 1983. Toxicity of phenthoate and changes in the organic constituents of the snail under sublethal and lethal impact. *Geobios.***10:** 250-253.

Reddy, R. D., Purushottam, K. R. and Ramamurthy, R. 1984. *J. Environ. Biol.* **5:** 119.

Siva Prasada Rao, K. Sathya Prasad K. and Ramana Rao, K. V. 1980. Sublethal effect of methyl parathion on tissue proteolysis in fresh water mussel, *Proc. Ind. Nat, Sci. Acad.* **346:** 164-167.

Trinder, C., Klin Chem Klin Boichem 1970. Clinical *Chemistry, Principles and Technics, 2nd Edition,* pp. 877-884.

Venkataramana, G. V., Sandya Rani, P. N. and Murthy, P. S. 2006. Impact of malathion on the biochemical parameters of gobiid fish. *J. Env. Biology.* 27(1): 119-122.

Yagana Banu, Sekh Amjad Ali and Tazia, Hameet 1981. Effect of sublethal concentration of DDT on muscle constituents of an air breathing cat fish,*Clariasbatrachus. Proc. Indian. Acad. Sci.* **90:** 33-37.

Young, D. S. 2000. Effects of drugs on clinical laboratory test ,*Fifth edition, AACC Press, Washington, D.C.* p. 1264.