



## TOXICITY OF ARSENIC ON GLYCOGEN CONTENT IN FRESHWATER FISH, *LABEO ROHITA* (HAM.)

**K. Pazhanisamy\* and N. Indra**

Department of Zoology, Annamalai University, Annamalai Nagar-608 002, Tamil Nadu, India

\*Present Address: Department of Zoology, Govt. Arts College, Ariyalur-621 713, Tamil Nadu

### ABSTRACT

The biochemical component like glycogen was estimated quantitatively in the tissues of liver and kidney of control and arsenic treated fish. The experimental fish were exposed to lower and higher sublethal concentrations (one-tenth and one-third of the 96 hr  $LC_{50}$  value) of arsenic for a period of 7, 14, 21 and 28 days. In both the tissues a significant reduction in glycogen level has been noticed. Maximum reduction was observed on the 28<sup>th</sup> day of exposure.

### INTRODUCTION

Toxicology is one of the most important multidisciplinary subjects in animal and plant sciences dealing with the adverse effects of chemicals and allied agents on living systems. It is also valuable in the protection of public health against hazards associated with toxic substances in food, air and water (Lu 1985). These toxic substances include heavy metals, pesticides, petroleum hydrocarbons, industrial chemicals and other inorganic compounds. Out of these, metal salts are considered to be the major source of pollution and are responsible for the ecological and environmental imbalance. The contamination of the environment due to an accelerated release of metal toxicants, has resulted in consequent hazards to animals and human health. These tissues have become issues of universal concern at the present moment (Singh 1985).

Most heavy metals are toxic to organisms as well as to human beings if the exposure levels are relatively high (Saikia et al. 1988). Among the various heavy metals, arsenic is possibly the most persistent in the environment and pose a threat to the aquatic species and has a detrimental effect on aquatic organisms, especially to fishes. The concentration of arsenic in the environment is of great concern as this element is recognized as a cumulative poison to humans and animals. As pollution may induce certain biochemical changes in fishes earlier to the manifestation of drastic cellular and systematic dysfunction, appropriate biochemical parameters could be used effectively as sensitive indicators (Aldridge 1983).

It can be inferred from several investigations that biochemical parameters could be effectively used to detect the effect of pollutants (Magendran 1990). Carbohydrate is an important energy source of all vital activities of an organism. It supplies a major portion of the energy required to the living system. Sastry & Gupta (1978) have pointed out the fluctuation in glycogen level in *Channa punctatus* when exposed to different concentrations of mercuric chloride. Ramakrishna & Sivakumar (1993) have reported the significant decline in glycogen content of liver tissues in *Oreochromis mossambicus* under toxic stress of Quinolphos. The fish *Tilapia mossambica* exposed to arsenic toxicity has resulted in the depletion of carbohydrate (Shobha et al., Rani 2000). The present study has been undertaken to investigate the changes in the glycogen content of the tissues of liver and kidney at different time intervals in the freshwater fish, *Labeo rohita* exposed to lower and higher sublethal concentrations of arsenic.

## MATERIALS AND METHODS

Healthy freshwater fish *Labeo rohita*, ranging from 10-12 cm in length and weighing 9-14g, were collected from fish farm located in Puthur, nearby Annamalai University, and acclimatized under laboratory conditions ( $29\pm 1^\circ\text{C}$ ) for a period of one week. The fish feed was provided everyday. The unused feed was renewed after 2 hours and water was renewed daily. The  $\text{LC}_{50}$  for arsenic trioxide for 96 hours was determined according to Finney (1971) and  $1/10^{\text{th}}$  of the  $\text{LC}_{50}$  value (0.27ppm) and  $1/3^{\text{rd}}$  of the  $\text{LC}_{50}$  value (0.91ppm) were taken as lower and higher sublethal concentrations respectively. The fish were exposed to these concentrations for treated and control period of 7, 14, 21 and 28 days. A control group was maintained at an identical environment. The fish were sacrificed from, both experimental and control groups, on 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> days of exposure periods and subjected to analysis of the biochemical changes. The glycogen content of the tissues, sampled from liver and kidney, was estimated by the method of Kemp & Andrienne Kits Van Heijninger (1954).

## RESULTS

The glycogen content estimated in the tissues of control and arsenic treated fish *Labeo rohita* are presented in Table 1.

**Liver:** In the control fish, the mean glycogen contents in the liver tissues were 14.51, 14.48, 14.51 and 14.49 mg/g wet wt. of tissue on 7, 14, 21 and 28 days of experimental periods respectively. In the fish treated with lower sublethal concentration of arsenic, the liver glycogen content was 13.05, 11.12, 9.38 and 8.05 mg/g wet wt. of tissue and in higher sublethal concentration, it was 11.00, 9.41, 7.53 and 5.02 mg/g wet wt. of tissue for 7, 14, 21 and 28 days exposure periods respectively. The per cent decrease with respect to the control was 10.06, 23.20, 35.35 and 44.44 in lower sublethal concentration, and 24, 19, 35.01, 48.10 and 65.35 in higher sublethal concentration for 7, 14, 21 and 28 days of exposure period respectively. The decrease in mean glycogen levels were statistically significant ( $p < 0.05$ ) in both sublethal concentration at all the exposure periods (Table 1).

**Kidney:** *Labeo rohita* treated with lower and higher sublethal concentration of arsenic exhibited a significant decrease in the mean glycogen content in the kidney tissues at all periods. The maximum reduction was noticed on 28<sup>th</sup> day of sublethal exposure to arsenic. The per cent decrease over the control was 12.50, 19.14, 23.40 and 35.41 in case of lower sublethal concentration of arsenic exposure. *Labeo rohita* when treated with higher sublethal concentration has also showed a gradual decrease in their glycogen content in kidney at all periods of intoxication. The maximum decrease was found on the 28<sup>th</sup> day of higher sublethal exposure to arsenic. The magnitude of decrease was high in higher sublethal concentration exposure period when compared to lower sublethal exposure period. The decreased level of glycogen at all periods of exposure was statistically significant (Table 1).

## DISCUSSION

In carbohydrate metabolism, glycogen plays an important role. The disturbance in glycogen profile was considered as one of the most outstanding lesions to the biological system due to the action of heavy metals (DeBurin 1976). The glycogen level of the liver and kidney tissues in fish *Labeo rohita* exposed to lower and higher concentrations of arsenic trioxide exhibits a decrease during the 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> day of exposure. The amount of glycogen in different tissues was less in treated fish than the control fish. This indicates that there is utilization of a major quantity of the glycogen

Table 1: Effects of lower and higher sublethal concentrations of arsenic on glycogen levels in liver and kidney tissues of *Labeo rohita* at different periods.

Tissues	Group	Exposure period in days				F Value
		7	14	21	28	
Liver	C	14.51±0.071	14.48±0.101	14.51±0.243	14.49±0.050	0.011 <sup>NS</sup>
	LC	13.05±0.043 (-10.06)	11.12±0.328 (-23.20)	9.38±0.194 (-35.35)	8.05±0.006 (-44.44)	133.10*
	HC	11.00±0.013 (-24.19)	9.41±0.008 (-35.01)	7.53±0.044 (-48.10)	5.02±0.002 (-65.35)	684.49*
Kidney	C	0.48±0.009	0.47±0.011	0.47±0.005	0.48±0.015	0.555 <sup>NS</sup>
	LC	0.42±0.0073 (-12.50)	0.38±0.0074 (-19.14)	0.36±0.0146 (-23.40)	0.31±0.0025 (-35.41)	47.34*
	HC	0.37±0.0025 (-22.91)	0.33±0.0051 (-28.26)	0.28±0.0027 (-37.77)	0.21±0.0024 (-55.31)	436.42*

C- Control; LC - Lower sublethal concentration; HC - Higher sublethal concentration; Mean ± S.E.- Indicates the mean of six individual observations; (+/-) indicates the per cent increase/decrease over the control; Values expressed in mg/g wet wt. of tissue; NS - Non significant; \* indicates significant at 5% level of F test

reserves and retention of less amount of glycogen content in the tissues. Liver and muscle are two active sites where storage and metabolism of glycogen takes place. In the present study a reduction in the glycogen content in the tissues can be attributed to the toxic effects of arsenic on tissue energy reserves.

Arockia Rita & John Milton (2006) have reported a decrease of carbohydrate content in liver, muscle, kidney and gills after exposure to carbamate pesticide in *Oreochromis mossambicus*. They have further reported that the consequence of increased glycogenolysis in fish under the stress of pesticide may be expressed as depleted levels of tissue carbohydrates. Verma & Tonk (1983) have recorded the decreased glycogen content in the liver and muscle of fish treated with mercury. A significant decrease in the level of glycogen content has been observed in muscle, intestine, brain and kidney tissues during the exposure of mercuric chloride in *Labeo rohita* (Jagadeesan 1994).

A diminution level of glycogen content in liver, gonad and muscle has been reported by Singh & Singh (2002) in *Channa punctatus* when exposed to aqueous latex extracts of *Euphorbia royleana*. Recently, Borah (2005) has also recorded continuing decline of glycogen in the tissue of liver and kidney of *Heteropneustes fossilis* exposed to petroleum oil. He has further suggested the glycogen content in both tissues indicating the excess utilization of carbohydrate to withstand pollution induced toxicosis. The carbohydrate reduction suggests the possibility of active glycogenolysis and glycolytic pathway to provide excess energy in stress conditions (Reddy et al. 1993). The median lethal dose of phosphomidan exhibited a steady and gradual decrease in the glycogen content of both liver and muscle tissue of *Polypedates maculatus* (Indra et al. 1998). The glycogen level of liver, muscle and kidney has shown a decrease in male and female *Rana cyanophlyctis* when exposed to 96 hours LC<sub>50</sub> concentration of dimethoate and methyl parathion (Mudgall & Patil 1987). Karuppasamy (1999) has found a significant decreasing trend in liver, muscle, gill and kidney glycogen content in *Channa punctatus* exposed to phenyl mercuric acetate. Moreover, he has stated that it may be due to increased glycogenolysis. A drop in glycogen content in kidney and intestine of *Anabas scandens* when exposed to lead nitrate showed the possibility of active genolysis (Mary Chandravathy & Reddy 1996).

The pattern of reduction in the total amount of glycogen revealed the greater utilization of carbohydrate resource and suggest the major contribution of carbohydrates towards the energy release during total calories depletion, and this corroborates with the earlier findings of Borah & Yadav (1995). Similarly, Ghosh (1986) has noticed the fall in the glycogen content in liver and kidney tissues of *Channa punctatus* and *Sarotheron mossambicus* exposed to dimethoate. Decrease in glycogen content in liver kidney and gill tissue was observed in *Oreochromis mossambicus* when exposed to ethofenprox (Muniyan 1999). Similar trend has been reported in *Clarias batrachus* exposed to malathion (Shoba Rani et al. 1991). Decrease in liver glycogen and glucose level has been reported in *Mystus vittatus* exposed to copper (Rajamanickam 1992). He has suggested that it may be due to increased glycogenolysis.

The present results suggest that glycogen being a ready source of energy, reduction in glycogen may be probably due to its more rapid breakdown or glycogenolysis which releases glucose in the circulatory system to meet needs of the increased energy requirements under a stressful situation posed by the heavy metal arsenic to *Labeo rohita*.

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## REFERENCES

- Aldridge, W.N. 1983. Mode of action, metabolism and toxicology. (Eds.) Miyamata and P.C. Keorney, Pergmon Press, New York, pp: 409-430.
- Arockia Rita, J.J. and John Milton M.C. 2006. Effect of carbamate pesticide Lannate (Methromyl) on the biochemical components of the freshwater cichlid *Oreochromis mossambicus* (Peters). Indian. J. Environ and Ecoplann., 12(1): 263-268.
- Borah, Sabita 2005. Effect of petroleum oil on biochemical constituents and enzyme activity in kidney and liver tissues of freshwater teleost fish, *Heteropneustes fossilis* (Bloch.) Nat. Env. Poll. Tech., 4(2): 227-232.
- Borah, S. and Yadav, R.N.S. 1995. Alteration in the protein, free amino acids, nucleic acids and carbohydrate contents of muscle and gill in rogor exposed freshwater fish, *Heteropneustes fossilis*. Pollution. Research, 15(3): 99-103.
- De Bruin, A. 1976. Biochemical toxicity of Environmental Agents. Elsevie. North-Holland - Bio Medical Press, Amesterdam.
- Finney, D.J. 1971. Probit Analysis. University Press, Cambridge, pp.333.
- Ghosh, T.K. 1986. Effect of dimethoate on tissue glycogen content of some freshwater fishes. Environ. Ecol., 4(4).
- Indra, N., Karpagaganapathy, P.R. and Meenakshi, V. 1998. Changes in glucose and glycogen level in blood and tissues of male tree frog, *Polypedates maculatus* (Gray) at median lethal dose (LD<sub>50</sub>) of phosphamidon. J. Natcon., 10(1): 119-122.
- Jegadeesan, G. 1994. Studies on the toxic effects of mercuric chloride and influence of antidote dimercaprol on selected tissues in *Labeo rohita* (Hamilton) fingerlings. Ph.D Thesis, Annamalai University.
- Karuppasamy, R. 1999. The effect of phenyl mercuric acetate (PMA) on the physiology, biochemistry and histology of selected organs in a freshwater fish, *Channa punctatus* (Bloch). Ph.D Thesis, Annamalai University.
- Kemp, A. and Andrienne Kits Van Heijininger, J.M. 1954. A colorimetric method for the determination of glucose and glycogen in tissues. Biochem. J., 56: 646-648.
- Lu, 1985. Basic toxicology fundamental, target organs and risk assessment. Hemisphere Publishing Corporation, Washington.
- Magendran, A. 1990. Studies of heavy metals (Cu, Cd and Hg) stress on pearlspot *Eroplus suratensis* (Bloch.). Ph. D. Thesis, Annamalai University.
- Mary Chandravathy, V. and Reddy, S.L. 1996. Lead nitrate exposure changes in carbohydrate metabolism of freshwater fish. J. Environ. Biol., 17(1): 75-79.
- Mudgall, C.F. and Patil, H.S. 1987. Toxic effects of dimethoate and methyl parathion on glycogen reserves on male and female *Rana cyanophyctics*. J. Environ. Biol., 8(3): 237-244.

- Muniyan, M. 1999. Effects of ethofenprox (trepon) on the biochemical and histological changes in selected organs of the freshwater fish *Oreochromis mossambicus* (Peters). Ph.D. Thesis, Annamalai University.
- Pramod Singh 1985. Environmental Pollution and Management. Cough Publication, India.
- Rajamanickam, C. 1992. Effects of heavy metal copper on the biochemical contents, bioaccumulation and histology of the selected organs in the freshwater fish, *Mystus vittatus* (Bloch). Ph.D. Thesis, Annamalai University, India.
- Ramakrishna, R. and Sivakumar, A.A. 1993. Effect of quinophos on the fish *Oreochromis mossambicus*. J. Ecobiol., 5: 45-50.
- Reddy, M.M., Kumar, V.A., Reddy, P.L.S and Reddy, S.N.L. 1993. Phenol induced metabolic alterations in the brain and muscle of freshwater fish *Channa punctatus* sublethal toxicosis. Journal of Ecotoxicology and Environmental Monitoring, 3(1): 7-11.
- Saikia, D.K., Mathur, R.P. and Srivastava, S.K. 1988. Heavy metals in water and sediments of upper Ganges. Indian J. of Environ. Hlth., 31(1): 11-17.
- Sastry, K.V. and Gupta, P.K. 1978. Effect of mercuric chloride on the digestive system of *Channa punctatus*. A histopathological study. Environ Res., 16: 270-278.
- Shobha Rani, A., Sudharsan, R., Reddy, T.N., Reddy, P.U.M. and Raju, T.N. 2000. Effect of sodium arsenite on glucose and glycogen levels in freshwater teleost fish, *Tilapia mossambica*. Poll. Res., 19(1): 129-131.
- Shobha Rani V.R., Venkateshwaralu, P. and Janaiah, C. 1991. Impact of sublethal concentration of malathion on certain aspects of metabolism in freshwater fish *Clarias batrachus* (Linn). Comp. Physiol. Ecol., 15(1): 13-16.
- Singh, Digvijay and Singh, Ajay 2002. Biochemical alteration in freshwater fish *channa punctatus* due to latices of *Euphorbia royleana* and *Jatropha gossypisolia*. Environmental Toxicology and Pharmacology, 12: pp. 129-136.
- Verma and Tonk, I.P. 1983. Effect of a sublethal concentration of mercury on the composition of liver, muscle and ovary of *Notopterus notopterus*. Water, Air and Soil Pollution, 29(3): 287-292.