Available online at www.journalijmrr.com

INTERNATIONAL JOURNAL OF MODERN RESEARCH AND REVIEWS

ISSN: 2347-8314

International Journal of Modern Research and Reviews

Volume 2, Issue 2, pp 74-78 February,2014

ORIGINAL ARTICLE

IMPACT OF ARSENIC METAL TOXICANT ON BIOCHEMICAL CHANGES IN THE GRASS CARP, *CTENOPHARYNGODON IDELLA*

S. Ananth, *V. Mathivanan, S. Aravinth and V. Sangeetha

Department of Zoology, Annamalai University, Annamalainagar – 608 002, Tamilnadu,
E-mail: mathivanandr@gmail.com

Article History: *Received 21st Jan,2014, Accepted 10th Feb,2014,Published 19th February,2014*

ABSTRACT

Arsenic is a pollutant widely distributed in nature and released into the environment through industrial processes and agricultural practices. Many countries have established various arsenic concentration limits for the protection of aquatic life. Arsenic can be found in both organic and inorganic compounds with variable oxidation states. Arsenic, known to cause alterations in various tissues of fish at the biochemical level. Glycogen is the main reserve source of energy for animals during normal metabolism and their content in tissue of fish exposed to chemical substances may indicate the health condition of the fish. The aim of the present study is to assess the glycogen content in, liver, gill, kidney and brain of the fish, *Grass carp* exposed to sublethal concentrations of arsenic trioxide of the 96 hrs LC_{50} values (89 mg/L) for the period of 7, 14, 21 and 28 days. The fish exposed to arsenic trioxide showed a decrease in the glycogen level for 7, 14, 21 and 28 days in liver, gill, kidney, and brain. The objective of the present work is to observe the effect of arsenic trioxide on glycogen levels in liver, gill, kidney and brain of Indian major carp, *Grass carp.*

Keywords: Arsenic trioxide, Glycogen, Freshwater fish *Grass carp*

1.INTRODUCTION

Arsenic is a naturally occurring, highly toxic environmental pollutant. It is present in water, soil and food and released into the environment from both natural and man-made sources (Tchounwou *et al.,* 1999). The drinking water containing more than 10 µg/L of arsenic is harmful to the body and chronic exposure to arsenic-contaminated water and food causes cancer (WHO, 2001). Arsenic is the first metalloid to be identified as a human carcinogen and most cases of chronic arsenic sis are associated with continual intake of arsenic-contaminated water (Jin *et al.,* 2004). Arsenic is used for alloying, as a catalyst in chemical reactors, for battery making and metal plating. It is deposited in rivers and streams through discharges of effluents from operations. It's route of exposure to humans, animals, and birds is through drinking contaminated water and dust from the atmosphere. Its health effects include; the disturbance of respiratory system and asthma, birth defects, vomiting and damage to Deoxyribonucleic Acid (DNA) at high concentrations (Ntengwe and Maseka, 2010).

Arsenic, an important environmental contaminant, is present in the aquatic environment as a result of geogenic and anthropogenic processes (Gonzalez *et al.,* 2006; Singh and Banerjee, 2008). In the environment, arsenic is present in different forms and the toxicity depends up on chemical form and oxidation states (Agusa *et al.,* 2008). Arsenic is classified as a metalloid with metallic and non-metallic properties. The most frequently used arsenic compound is arsenic trioxide (As_2O_3), which is used for agricultural chemicals and synthesis of various inorganic and organic compounds (Buchet *et al.,* 1994; Juma *et al.,* 2002) have shown that fish is the major source of exposure of arsenic and humans who consume these tissues may be threatened by arsenic toxicity (Lin *et al.,* 2005). Fish tissues, skin, nervous system, gastrointestinal system and blood are commonly involved in arsenic poisoning (ATSDR, 2006). Hence, the utility of fish in assessing contaminations in water has gained prominence in recent years (Ikem and Egiebor, 2005; Yilmaz and Yilmaz, 2007).

In the aquatic environment, arsenic (metalloid element) found either as arsenite (As^{3+}) or arsenate (As^{5+}) form which are inter-converted through redox and methylation reactions (Bears *et al.,* 2006). The arsenic species can accumulate in many aquatic organisms which may catalyse the oxidation of arsenite to arsenate and also promote the formation of methylarsines through biomethylation reaction (Ridley *et al.,* 1977). The aquatic environment, fish are usually regarded as organisms of choice for assessing the effects of environmental pollution on aquatic ecosystem (Gernhöfer *et al.,* 2001), because fish can be chronically exposed to different substances, such as heavy metals and pesticides, and

***Corresponding author:** Dr. V.Mathivanan, Department of Zoology, Annamalai University,Annamalainagar -608002,Tamilnadu*

they can bio-accumulate by direct exposure or through the food chain. In fish, gills are critical organs for respiratory and osmoregulatory functions. Gills are generally considered as the good indicators of water quality, being models for studies of environmental impact because gills come into immediate contact with the environment and are the primary route for the entry of arsenic and other pollutants. Furthermore, any damage to this important organ can compromise fish survival (Palaniappan *et al.*, 2008).

Fish can serve as bioindicators of environmental pollution and can play significant roles in assessing potential risk associated with contamination in aquatic environment since they are directly exposed to chemicals resulting from agricultural production via surface run-off or indirectly through food chain of ecosystem (Lakra and Nagpure, 2009). Carbohydrates play a structural role as well acts as a reservoir of chemical energy to be increased or decreased according to organisms need. Animals store homopolysaccharides in tissue as glycogen consisted of glucose and is considered to be the major source of energy and hence all metabolic events depend upon the breakdown of glycogen. Glycogen in the tissue is also considered to be the immediate source of energy to adapt to the environmental conditions. Several workers have reported the impact of various heavy metals on the carbohydrate metabolism of different aquatic organisms (Kharat *et al.*, 2009). Heavy metal- copper is an osmoregulatory toxicant in Gibel carp, *Carassius auratus* causing Na loss and glycogen depletion in liver (Boeck, 2010).

Glycogen is the main reserve source of energy for animals during normal metabolism and their content in liver and muscle of fish exposed to chemical substances may indicate the health condition of the fish. During unfavourable environmental situation, the normal metabolism is affected which in turn leads to alteration in the glycogen reserve of fish. The observed reduction in glycogen content in the present study indicates the utilization of stored glycogen to meet out the high energy requirement under the lindane stress. Similar reduction in glycogen content in *Clarias batrachus* was observed after fish were exposed to pesticide (Begum and Vijayaraghavan, 1996; Moorthikumar and Muthulingam, 2011). The depletion of glycogen level clearly indicates its rapid utilization to meet the enhanced energy demands in fish exposed to toxicant through glycolysis or Hexose Monophosphate pathway. It is assumed that decrease in glycogen content may be due to the inhibition of hormones which contribute to glycogen synthesis (Sobha *et al.*, 2007). They showed that these fishes were, hypoglycemic ,hypolactemic and the total plasma proteins, the levels of glycogen, lactic acid, pyruvic acid and total proteins in liver and muscles decreased significantly in both acute and chronic exposure. However, no information is on record concerning the sublethal concentration of heavy metal arsenic trioxide effect on the glycogen levels of *Grass carp*.

2.MATERIALS AND METHODS

The fish *Grass carp* having mean weight 15-17 gm and length 14 – 16 cm were collected from PSP fish farm, at Puthur and acclimatized to laboratory conditions. They were given the treatment of 0.1%$KMNO_4$ solution and then kept in plastic pools for acclimatization for a period of nine days. They were fed on rice bran and oil cake daily. The arsenic trioxide was used in this study and stock solutions were prepared. Arsenic trioxide LC_{50} values were 89 mg/L respectively taken as sublethal concentrations for this study. Fifty fish were selected and divided into 5 groups of 10 each. The first group was maintained in free from arsenic trioxide and served as the control. The other 4 groups were exposed to sublethal concentration of arsenic trioxide in 10 litre capacity aquaria. The 2^{nd}, 3^{rd}, 4^{th} and 5^{th} groups were exposed to arsenic trioxide for 7, 14, 21 and 28 days respectively. At the end of each exposure period, the fish were sacrificed and the required tissues were collected for glycogen estimation. The glycogen content of the tissues was estimated by the method of Kemp and Kits Van Heijininger (1954).

Statistical Analysis

The data were analyzed by applying Analysis of Variance two way ANOVA to test the level of significance. Results were presented as means ± SE. P values < 0.05 were regarded as % changes over the control to student 't' test the level of significance (Duncan, 1957).

3.RESULTS

The glycogen levels in gill, liver, kidney, and brain of *Grass carp* exposed to sublethal concentration of heavy metal Arsenic trioxide showed significant decrease when compared to control fish. The decrease in gill, liver, kidney and brain of *Grass carp* glycogen levels were more pronounced at 28 days of exposure periods (Table 1).

4.DISCUSSION

The arsenic concentration in natural water bodies mainly depends on geological composition and the degree of pollution (Jain and Ali, 2000). The concentration of arsenicals like As^{3+} and As^{5+} in the aquatic environment mostly depends on the valance state, redox conditions and geological environment (Suhendrayatna *et al.*, 2002. The impact of arsenic toxicity is mostly controlled by physico chemical properties of water rather than by their total concentration (Suhendrayatna *et al.*, 2002). Arsenic has definite role in depletion of carbohydrate store which might be a counter active mechanism to fight and survive under toxic environment. Apart from arsenic, other heavy metals and pollutants like pesticides also alter the biochemical composition of different organs. Parvathi *et al.*, 2011 observed alteration in the biochemical composition in different tissues of freshwater fish, *Cyprinus carpio*. Decrease in the glycogen content may be due to enhanced breakdown of glycogen to glucose through glycogenolysis in the fish tissues to withstand the existing stress condition, mediated by catecolamine and adenocortical harmones (Gluszak *et al.*, 2007).

The depletion of glycogen from different tissue system of *C. batrachus* following exposure to trivalent arsenic might be due to enhanced utilization of the glycogen as the immediate source to meet the energy requirement under arsenic stress. According to Kumari and Ahsan (2011), depletion of glycogen in the liver and kidney suggests that these tissues do not contribute much anoxia resulting from resulting from pollution stress, since anoxia and hypoxia are known to increase carbohydrate consumption or may be due to

generalized disturbances in carbohydrate consumption Simon *et al.*, 1983). These alterations may be due to rapid utilization of glycogen to meet the energy demands under stress condition and supply energy demand in the form of glucose which undergoes breakdown to produce energy rich compound ATP through glycolytic pathway as suggested by utilization of glucose to meet increased metabolic cost as suggested by Viswarajan *et al*. (1988) in *Oreochromis mossambicus* under the stress of tannic acid. Decrease in liver glycogen may also be due to acute hypoxia (Heath and Pritchard, 1965). The decreased level of glucose and

Table 1. The level of glycogen in the selected tissues of freshwater fingerlings *Grass carp* exposed with sublethal concentration of arsenic trioxide.

		7 days	14 days	21 days	28 days
Liver	15.54±0.38	12.07±0.42	9.42±0.37	6.13±0.27	4.18±0.17
		-22.32	-39.38	-60.55	-73.10
%COC		6.30*	12.00*	20.91*	28.40*
Gill	9.57±0.33	8.45±0.25	6.59±0.29	4.93±0.19	2.71±0.10
		-11.70	-31.13	-48.47	-71.68
%COC		2.87*	7.26*	12.88*	20.17*
Kidney	7.31±0.25	6.48±0.29	4.12±0.20	3.36±0.13	2.59±0.10
		-11.35	-43.62	-54.03	-65.56
%COC		2.51*	11.00*	15.19*	18.15*
Brain	11.94±0.31	8.13±0.20	7.39±0.25	5.20±0.21	3.17±0.12
		-31.90	-38.10	-56.44	-73.45
%COC		10.88*	12.29*	19.20*	27.40*

The values are mean ± S.E six individual observations. (Values are expressed as mg/g wet wt. of tissue).
*significant (P<0.05) of student 't' test. % COC – Percent change over the control.

Omkar *et al.* (1984). Similar results were obtained by (Muley *et al.*, 2007). The study reveals that zinc has a tangible effect on the glycogen and protein level of certain tissues of freshwater fish, *Channa gachua*, which may cause severe to fatal physio-metabolic dysfunction.

The results of the present study showed that the sublethal concentrations of heavy metal arsenic trioxide significantly altered the glycogen levels in liver, gill, kidney, and brain of *Grass carp* after 7, 14, 21 and 28 days exposure. The glycogen levels were decreased in the liver, gill, kidney, and brain of *Grass carp* when exposed to sublethal concentrations of arsenic trioxide may be glycogenolysis takes place by the action of heavy metal arsenic trioxide. A fall in glycogen levels clearly indicates its rapid utilization to meet the enhanced energy demands in pesticide treated individuals through glycolysis or hexose monophospahte pathway (Cappon and Nicholes, 1975). Decreased glycogen synthesis is attributed to inhibition of enzyme glycogen synthesis (Stamp and Lesker, 1967). The decreased glycogen concentration in the liver of common carp could be due to its enhanced utilisation as an immediate source to meet the energy demand under metallic stress. Depleted glycogen level under chromium stress reported in *Labeo rohita*, Vutkura, (2005) has also supports these findings. The carbohydrate source is stored as a reserve fuel in the liver and muscle tissues of fish for the endogenous derivation of energy during acute and chronic stress (Bonga, 1997).

The decreased glycogen content as a result of hypoxic or anoxic condition activates the glycolytic enzymes via catecholamines that initially enhance glycogen concentration. It was also found that cadmium could decrease glycogen reserves in the liver and muscle tissues of *Cyprinus carpio* (Cicik and Engin, 2005). The glycogen level however decreased subsequently on further continuation of exposure. Thirumavalavan and Samipillai, (2010) also noticed decreased level of glycogen in the brain of *Catla catla*, exposed for 21 days to 0.1mg/L of arsenic trioxide. Decrease in carbohydrates is probably due to glycogenolysis and glycogen contents in the liver, muscle, intestine, kidney and brain of *Channa punctatus* exposed to phenyl mercuric acetate (Karuppasamy, 2000; Moorthikumar and Muthulingam, 2011). Heavy metals arsenic trioxide thus may produce damage to an organ, inhibition of enzymes activity and significant alterations in various metabolic activities.

5.CONCLUSION

In conclusion, this study showed that arsenic trioxide altered the carbohydrate metabolism in the freshwater fish *Grass carp* by affecting the levels of glycogen in liver, gill, kidney, and brain due to impairments in energy requiring vital processes.

6.ACKNOWLEDGEMENT

The authors wish to thank the authorities of Annamalai University for providing the facilities to carry out this study.

7.REFERENCES

Agency for Toxic Substances and Disease Registry (ATSDR), 2006. Toxicological profiles for Arsenic. Atlanta, USA.

Agusa, T., Takagi, K., Kubota, R., Anan, Y., Iwata, H. and Tanabe, S. 2008. Specific accumulation of arsenic compounds in greenturtles (Cheloniamydas) and hawksbill turtles (*Eretmochelys imbricata*) from Ishigaki Island, Japan. *Environ. Pollut*. 153, 127–136

Bears, H., Richards, J.G. and Schulte, P.M. 2006. Arsenic exposure alters hepatic arsenic species composition and stress mediated-gene expression in the common killifish (*Fundulus heteroclitus*). *Aquat. Toxicol*. 77, 257–266.

Begum, G and Vijayaraghavan, S. 1996. Alterations in protein metabolism of muscle tissue in the fish Clarias batrachus (Linn) by commercial grade dimethoate, *Bull. Environ. Contam. Toxicol.*, 57 : 223–228.

Boeck De, G., Smolders, R. and Blust, R. 2010. Copper toxicity in gibel carp, *Carassius auratus*: Importance of sodium and glycogen. *Comp. Biochem. Physio*.C.*Toxicol.Pharmacol*.152 (3):332-337.

Bonga S. E. W., "The stress response in fish", Physiol. Rev., 1997. 77, 591-625.

Buchet, J.P., Pauwels, J. and Lauwerys, R. 1994. Assessment of exposure to inorganic arsenic following ingestion of marine organisms by volunteers. *Environ. Res*. 6, 44–51.

Cappon, I.D. and Nicholes, D.M. 1975. Factors involved in increased protein synthesis in liver microsomes after administration of DDT. *Pestic. Bio Chem. Physiol. 5:* 109-118.

Cicik, B and Engin, K.2005. "The effects of cadmium on levels of glucose in serum and glycogen reserves in the liver and muscle tissue of *Cyprinus carpio* L.", Turk. J. Vet. *Anim. Sci*., 29: 113-117.

Duncan, B.D. 1957. Multiple range tests for correlated and heteroscedastic means. *Biometrics*, 13: 359-364.

Gernhofer, M., Pawert, M., Schramm, M., Müller, E. and Triebskorn, R. 2001. Ultrastructural biomarkers as tools to characterize the health status of fish in contaminated streams. *J. Aquat. Ecosyst.* Stress Recov. 8, 241–260.

Gluszak, L., Miron Dios, S., Mores, B., Simoes, R., Schetinger, M., Morch, V. and Loro V. 2007.Acute effect of glyphosate herbicide on metabolic and enzymatic parametersof Silver Catfish *Rhambia quelen.Comp Biochem Physiol C Toxicol Pharmacol*.,146:519-524.

Gonzalez, H.O., Roling, J.A., Baldwin, W.S. and Bain, L.J., 2006. Physiological changes and differential gene expression in mummichogs (*Fundulus heteroclitus*) exposed to arsenic. *Aquat. Toxicol.* 77, 43–52.

Heath, A.G and Fritechard, A.W. 1965. Effect of severe hypoxia on carbohydrate energy, stores and metabolism in two species of freshwater fish. *Physiol. Zool.*, 38, 325-334.

Ikem, A. and Egiebor, N. 2005. Assessment of trace elements in canned fishes (mackered tuna, Salmon, Saruies and Herrings) marketed in Georgia and Alabama (USA). *J. Food compos. Anal*. 18, 771–787.

Jain, C.K. and Ali, I., 2000. Arsenic: occurence, toxicity and speciation techniques. *Water. Res*. 34, 4304–4312.

Jin, Y., Sun, G., Li, X., Li, G., Lu, C. and Qu, L. 2004. Study on the toxic effects induced by different arsenicals in primary cultured rat astroglia. *Toxicol. Appl. Pharmacol*. 196, 396–403

Juma, H., Battah, A., Salim, M. and Tiwari, P. 2002. Arsenic and Cadmium levels in imported fresh and frozen fish in Jordan. *Bull. Environ. Contam. Toxicol*. 68, 132–137.

Karuppasamy, R. 2000. Effect of phenyl mercuric acetate on carbohydrate content of *Channa punctatus* Uttar Pradesh *J. Zool*., 20(3) : 219-225.

Kemp, A. and Kitsvan Heijhinger, J.M. 1954. A colorimetric micromethod for the determination of glycogen in tissues. *Biochem. J.*, 56: 646-648.

Kharat, PS., Ghoble, LB., Shejule, K.B. and Ghoble, BC.2009. Effect of TBTCL on Glycogen profile in Fresh water Prawn, *Macrobrachum kistensis*. *World Applied Science Journal* 7(12):1534-1539.

Kumari, B. and Ahsan, J. 2011. Study of muscle glycogen content in both sexes of an Indian teleost *Clarias batrachus* (Linn.) exposed to different concentrations of Arsenic. *Fish Physiology and Biochemistry*, 37:161-167.

Lakra, W.S and Nagpure, N.S. 2009. Genotoxicological studies in fishes: A review. Indian J. Anim. Sci. 79 : 93- 98.

Lin, M.C., Lin, H.Y., Cheng, H.H., Chen, Y.C. and Liao, C.M. 2005. Risk assessment of arsenic exposure from consumption of cultured Milkfish, Chanos chanos (Forsskal), from the arsenic-contaminated area in Southwestern Taiwan. *Bull. Environ*. *Contam. Toxicol*. 75, 637–644.

Moorthikumar, K. and Muthulingam, M. 2011. Potential use of glycogen contents as biomarker of Nickel chloride stress on Indian major carp *Labeo rohita* (Hamilton). *Asian Journal of Science and Technology Vol. 1, Issue 4, pp.079-084.*

Muley, D.V., Karanjkar, DM. and Maske, SV.2007. Impact of industrial effluents65Muley on the biochemical composition of Fresh water fish *Labeo rohita. Biol*.28 (2)245-249.

Ntengwe, F.W. and Maseka, K.K. 2010. The impact of effluents containing zinc and nickel metals on stream and river water bodies: the case of chambishi and mwambashi streams in *Zambia. Journal of Environmental Sciences*, 42 : 321 – 327.

Omkar, R.K. and Shukla, G.S. 1984 .*Acta Hydrochi hydrobiol.German*. 12 (5):549.

Palaniappan, PL.RM. and Vijayasundaram, V. 2008. Fourier transform infrared study of protein secondary structural changes in the muscle of *Labeo rohita* due to arsenic intoxication. *Food Chem. Toxicol*. 46, 3534–3539.

Parvathi, K., Palanivel, S., Mathan, R. and Saras, R.2011. Sublethal effects of Chromium on some biochemical profiles of Fresh water Teleost, *Cyprinus carpio.I nternat*. *J. Appl.Biol.and Pharmasutical Tehc*.295-300.Vol.2

Ridley, W.P., Dizikes, L.J. and Wood, J.M. 1977. Biomethylation of toxic elements in the environment. Science 197, 329–332.

Simon, L.M., Nemcsok, J, and Boross, L.1983. Studies on the effect of paraquate on glycogen mobilaztion in liver of Common carp *Cyprinus carpioL.Comp Biochem Physiol* 75C(1) :167-169.

Singh, A.K. and Banerjee, T.K. 2008. Toxic effects of sodium arsenate (Na_2HAsO_4_$7H_2O$) on the skin epidermis of air-breathing catfish *Clarias batrachus* (L.). *Veterinarski Arhiv*. 78 (1), 73–88

Sobha, K., Poornima, A., Harini, P and Veeraiah, K. 2007. A study on biochemical changes in the fresh water fish, Catla catla (Hamilton) exposed to the heavy metal toxicant cadmium chloride. Kathmandu University Journal of Science, Engineering and Technology, 1(4): 1-11.

Stamp, W.D. and Lesker, P.A. 1967. Enzyme studies related to sex difference in mice with hereditary musculardystrophy. *American Medical J. Physiol.*, 213:587.

Suhendrayatna, O.A., Nakajima, T. and Maeda, S. 2002. Studies on the accumulation and transformation of arsenic in freshwater organisms. I. Accumulation,

transformation and toxicity of arsenic compounds on the Japanese medaka, Oryzias latipes. *Chemosphere* 46, 319–324.

Tchounwou, P.B., Wilson, B.A. and Ishaque, A., 1999. Important considerations in the development of public health advisories for arsenic and arsenic containing compounds in drinking water. Rev. *Environ. Health* 14, 1–19.

Thirumavalavan, R. and Sankar Samipillai, S. 2010. Arsenic induced biochemical changes in the brain tissue of fresh water fish, *Catla catla. International Journal of Current Research*, 1: 30-34

Viswarajan, S and Muthukrishnan. S. 1988. Impact of tannery effluent on phosphatase activity of fishes. *Proc. Inds. Nat. Sci. Acd*. B 55 314-321.

Vutkuru, S.S.2005 "Acute effects of hexavalent chromium on survival, oxygen consumption, hematological parameters and some biochemical profiles of the Indian Major Carp, *Labeo rohita*", *Int. J. Environ. Res. Public Health*,2: 456-462

WHO, 2001. Environmental Health Criteria 224. Arsenic and Arsenic Compounds, 2nd ed. World Health Organization,Geneva. http://www.whqlibdoc.who.int/ehc/WHO_EHC_224.pdf>.

Yilmaz, A.B. and Yilmaz, L. 2007. Influences of sex and seasons on levels of heavy metals in tissues of green tiger shrimp (*Penaeus semisulcatus* de Hann, 1844). *Food Chem*. 101, 1664–1669.