

ALTERATIONS IN THE CENTRAL DOGMA UNITS UNDER TRIVALENT ARSENIC STRESS DURING PREPARATORY PHASE OF OVARIAN CYCLE OF MYSTUS (M.) VITTATUS (BL.)

Anuradha Shukla, Yogendra Kumar Payasi* & J.P. Shukla*

P.G. Department of Zoology

SH. Kisan P.G. College, BASTI (U.P.)

*IGNTU (A Central University) Amarkantak, 484 887 (M.P.), India.

ABSTRACT : Central dogma units comprise of DNA, RNA and synthesis of protein. Reproductive cycle in fishes is always dynamic and the units of central dogma are always changing in different phases of testicular and ovarian cycle which constitute reproductive cycle in fishes. The adult specimens of *Mystus (M.) vittatus*, a tropical siluroid, when exposed for 30 days to sublethal concentration of trivalent arsenic (11.24 mg/l) revealed significant decline in DNA, RNA and consequently protein in the ovary of *Mystus (M.) vittatus* during its preparatory phase. However, 15 days exposure revealed less significant alterations. Causes for declining in various units of central dogma and consequently protein synthesis is discussed in this paper.

KEYWORDS : DNA; RNA; Protein; *Mystus (M.) vittatus* ; Preparatory phase; Ovarian cycle.

INTRODUCTION

Heavy metal pollution at present has become a serious environmental and public health hazard. It is because, the concentration of metallic pollutants released into the different section of environment from various industrial processes. These are often concentrated because of their bio-accumulative and non-biodegradable features. Heavy metals constitute a core group of aquatic pollutants^{26,27}. Their high toxicity even at low concentration may exert cumulative toxic effects in a wide variety of fish fauna and other aquatic biota²³. The introduction of heavy metals into the environment is through a wide spectrum of natural sources such as volcanic activities, erosion, and anthropogenic ones, including industrial wastes release as well as leakage. Certain metallic pollutants such as

chromium, arsenic, nickel, cadmium, mercury etc. exert toxic effects on living biota even at low concentration whereas zinc, manganese, copper etc. produce toxic effects on living biota only at higher concentration^{4,14,28}. In the aquatic environment, fishes appears to be remarkable bioindicator of arsenic toxicity^{10,12}. Allen and Rana², reported that toxicity of arsenical compounds depends upon species, sex, age, dose, duration of exposure, organic or inorganic form, valency state etc. Arsenic has been reported to be present in two different oxidation states (+3 and +5). Trivalent arsenic (As^{+3}) has been observed to be more deleterious than the pentavalent arsenic^{3,12,15,21,22}. Even though, the toxicity of arsenic (+3) in aquatic biota, particularly fishes has been enormously documented^{21,22,23,25}, however, impact of trivalent arsenic on the units of central

dogma in ovarian cycle phases is scarce and hence present study has been undertaken.

MATERIALS AND METHODS

Adult specimens of *Mystus (M.) vittatus* (Bl.) were collected from local lake having weight 92.38 ± 4.48 gm. They were acclimatized in laboratory tap water having pH = 7.2 ± 0.02 ; temperature = $22.4 \pm 2.2^\circ\text{C}$; DO = 6.2 ± 0.52 mg/l; hardness as CaCO_3 = 126.62 ± 3.6 mg/l. Analysis of physico-chemical features of tap water was made by the methods outlined by APHA¹. Fish were acclimatized for 10 days in tap water during preparatory phase (January to April). Sublethal concentration (11.24 mg/l) of trivalent arsenic (one tenth of LC_{50} 96 hours) was selected for long term experimentation (30 days) during preparatory phase of ovarian cycle of *Mystus (M.) vittatus* as outlined by Shukla and Pandey²⁰. The control and experimental media was aerated 3-5 hours daily using stone diffusers, though *Mystus (M.) vittatus* is hardy air breathing fish.

The biochemical estimation of the key units of central dogma (DNA and RNA) in the testis during its preparatory phase was made using the methods adopted by Schneider²⁴.

The protein concentration in the testis during preparatory phase was measured using the method proposed by Lowery *et al.*¹⁷. The data obtained in our study was statistically analyzed for significant by Student's 't' test as proposed by Fischer⁷, and a p value of 0.05 or less

was noticed as significant between control and experimental.

RESULTS AND DISCUSSION

The DNA and RNA content in the control group during preparatory phase of testicular cycle was 48.46 ± 0.14 and 84.48 ± 0.32 $\mu\text{g}/100\text{mg}$ wet weight of testis where as protein content was 94.22 ± 0.66 mg/gm wet weight of testis. Poorly significant decrease was noticed in these units after 15 days of exposure to SLC of trivalent arsenic. However, significant decrease was noticed after 30 days of exposure in experimental media showing exposure duration dependent alterations.

It is a well-known fact that oogenesis which occurs in ovary is a dynamic event and starts during preparatory phase of ovarian cycle. Available literatures reveal that arsenical compounds inhibit the DNA synthesis by producing a number of chromosomal abnormalities^{5,9,19}. Further, Peters *et al.*¹⁸ and Fowler⁸, reported that arsenical compounds cause chromosomal abnormalities by simply substituting for phosphate which form phosphodiester bonding in the DNA chain and prove teratogenic. Reports of Lolyd¹⁶, Farag *et al.*,⁶ reveal that metallic toxicants bring intra-strand cross links and strand breaking in fishes and hence decrease in the DNA content takes place. Significant decline in RNA content after 30 days of exposure under trivalent arsenic stress during preparatory phase may be attributed to the fact that the enzymes responsible for

Table-1. Nucleic acids (DNA and RNA) and protein in the ovary of *Mystus (M.) vittatus* during preparatory phase exposed for 15 and 30 days under SLC of trivalent arsenic compared with control. Each value represents mean $\pm$ SE of 5 observations

Content	Control	15 days exposure	% alterations	30 days exposure	% alterations
DNA(μ g/100mg) of wet testis	56.26 $\pm$ 0.48	54.44 $\pm$ 0.62 ⁰⁰	-3.23	49.36 $\pm$ 0.36 ^{**}	12.31
RNA(μ g/100mg) of wet testis	98.46 $\pm$ 0.44	94.52 $\pm$ 0.52 ⁰⁰	4.00	88.22 $\pm$ 0.66 ^{**}	10.40
Protein(mg/gm) of wet testis	106.28 $\pm$ 0.88	98.36 $\pm$ 0.92 [*]	9.90	92.20 $\pm$ 0.82 ^{***}	13.24

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; 00 = > 0.05

transcription may be damaged, hence quantitative decline in the RNA content during preparatory phase of ovarian cycle of *Mystus (M.) vittatus* becomes obvious. Also it is possible that arsenical components may interfere in the transcription process causing quantitative decline in RNA.

The decline in the protein content under SLC of trivalent arsenic after long-term exposure (30 days) may be because of its interference in nucleic acids metabolism as shown in Table 1, hence quantitative decrease in protein content becomes obvious. Also, trivalent arsenic may inactivate the intracellular protein and may also block the metabolism of proteogenic amino acids whose number is 20. As a result, decrease in protein content during preparatory phase of ovarian cycle becomes obvious.

ACKNOWLEDGEMENT

Authors (A. Shukla & J.P. Shukla) thank to CST (UP) for financial assistance

vide letter no. CST/D-265 dt. 14.5.2015.

REFERENCES

1. APHA, 2005. Standard Methods for examinations of water and waste water, 21st Edition, Washington DC.
2. Allen, T. and S.V. Rana, 2004. Effect of arsenic on glutathione dependent enzymes in liver and kidney of fresh water fish *Channa punctatus*. *Biol. Trace. El. Res.*, 100:39-48.
3. Bears, H.R., J.G. Chards and P.M. Chitti, 2006. Arsenic exposure alters hepatic and stress mediated gene expression in the common killfish, *Fundulus heteroclitus*. *Aquat Toxicol.*, 77:257-266.
4. Cohen, T., S. Hee and R. Ambrase, 2001. Trace metals in fish and invertebrate of three California coastal wetlands. *Mal. Pollu. Bull.*, 42: 224-232.
5. Dikshith, T.S.S., 1973. In vivo effects of parathion on guinea pig chromosomes. *Environ. Physiol. Biochem.*, 3:161-168.
6. Farag, A.M., T. May, G.D. Marty, M. Easton, D.D. Harpner, E.E. Little and L. Cleveland, 2006. The effect of chronic chromium exposure on the health of chiwok salmon (*Oncorhynchus tshawytscha*). *Aquat. Toxicol.*, 76(3):246-257.

7. Fischer, R.A., 1983. Statistical methods for research workss. 13 Eds. Oliver and Boyd, London, pp. 122-125.
8. Fowler, B.A., 1977. Toxiology of environmental arsenic. In: Toxiology of trace elements. Washington, Hemisphere Pus. Corpn., 2: 78-122.
9. Freed, J.J. and S.A. Sehetzis, 1969. Chromosomes aberration in cultured cells deprived of single essential amino acids. *Exp. Cell. Res.*, 55:393-397.
10. Gerofer, M., M. Powert, M. Schramm, Muller and R. Riebskorn, 2001. Ultra-structureal bio-markers as tools to characterize the health states of fish in contaminated streams. *J.Aqua. Ecosyst. Stress Res.*, 8:241-260.
11. Ghosh, D., S. Bhettacharya Mazumdar, 2006. Purturbations in cat fish immune response by arsenic, organ and cell specific effects. *Comp. Pharmacol. Toxicol S.*, 143:455-463.
12. Ghosh, D., S. Bhettacharya and S. Mazumdar, 2007. Long term exposure to arsenic effects to head kidney and impair humoral immune response of *Clarias Satrachus*. *Aquat. Toxicol.*, 81: 79-89.
13. Harper, A.H., 1983. Review of biochemistry. 20th Eds. Large Medical Publication Co. California, pp. 1012.
14. Karadore, A.H. and E. Unlu, 2007. Heavy metal concentration in water, sediment, fish and some benthic organism from Tigris river, *Turkey Env. Monit. Assess.*, 131:323-327.
15. Kovandon, K.S., S. Janansthana and M. Saranaman, 2013. Expression of malathion in liver and Kidney of freshwater Vineer fish, *cyprinus carpio* var *communis* (Linn) exposed to arsenic. trioxide *Ame J. Sci. Indus. Res.*, 4(1):1-10.
16. Lolyd, D.R., D.H. Phillips and P.L. Carmichad, 1997. Generation of purative intra-strand cross-link and strand break in DNA by transition metal ion mediated oxygen radical attack. *Chem. Res. Toxicol.*, 10:393-400.
17. Lowery, O.H., N.J. Rosebrough, A.L. Furr and R.J. Randall, 1951. Cited in colowick SP and kaplon NO. Eds. Methods in enzymology. 3: 448-450.
18. Peters, J., V. Salmoid and W.U. Kand, 1970. Chromosome aberration chilchen lymphosystem. *Med. Wo. Ch.*, 95:79-80.
19. Palmer, K.A., S. Green and M.S. Legator, 1972. Cytogenetic effects of DDT and derivative of DDT in cultural Mammalian cell line. *Toxicol. Appl. Pharmacol.*, 22:355-362.
20. Shukla, J.P. and K. Pandey, 1988. Toxicity and long-term effects of a sublethal concentration of cadmium on the growth of the fingerlings of *ophiocephalus punctatus* (Bl.). *Acta Hydrogen. Hydrobiol.*, 16(5): 537-540.
21. Shukla, Anuradha and J.P. Shukla, 2016. Toxic impact of arsenic on the blood pyruvate level in the fingerlings of a freshwater siluroid, *Mystus (M.) vittatus* (Bl). *The Gl. J. Env. Sci. & Res.*, 3:59-62.
22. Shukla, Anuradha and J.P. Shukla, 2017. Succinate dehydrogenase activity as an index of trivalent arsenic toxicity in fingerlings of tropical fresh water siluroid *Mystus (M) vittatus* (Bl). *Int. J. Curr. Trends in Sci. & Tech.*, 8(1):20482-20486.
23. Storelli, M.M., G. Isabarove, A. Storelli and G.O. Macrotrigiano, 2006. Trace metals in tissue of Mugilids (*Mugil aratus*, *Mugil capito* and *Mugil labrus*) from the Mediterranean sea. *Bull. Env. Conam. Toxicol.*, 77: 43-50.
24. Schneider, W.C., 1945. Phosphorus compounds in animal tissues. Extraction and estimation of deoxypentose nucel acid and pentose nucel acid. *J. Biol. Chem.*, 161:293-303.
25. Vankataramraddy, V., S.S. Vutukutu and P.B. Tchounnam, 2009. Ecotoxicology of hexavalent chromium in freshwater fish. *Rev. Environ. Health*, 24(2): 129-145.
26. Vutukur, S.S., 2003. Chromium induced alterations in some biochemical profiles of the Indian major carp, *Labao rohita* (Ham). *Bull. Environ. Contam Toxicol.*, 70(1): 118-123.
27. Vutukur, S.S., 2005. Acute effects of hexavalent chromium on survival, oxygen consumption,

Σ

- hematological parameters and some biochemical profiles of Indian major carp, *Labeo rohita*. *Int. J. Environ. Res. Public Health*, 2(3): 456-462.
28. Yilmaz, A.B., T. Cemal and T. Tashin, 2010. Uptake and distribution of hexavalent chromium in tissue gill, skin and muscle of a freshwater fish, Tilapia, (*Oreochromis aureus*). *Env. Chem & Ecotoxicol*, 2(3): 28-33.