IOSR Journal of Engineering (IOSRJEN) **www.iosrjen.org**
ISSN (e): 2250-3021, ISSN (p): 2278-8719
Vol. 07, Issue 12 (December. 2017), ||V1|| PP 05-09

Sodium arsenite induced histochemical changes in the liver of fish *Mystus bleekeri* (Day)

Borale Rajendra P* and Khodake Sanjay P.
Jaihind Educational Trust's Z. B. Patil College, Deopur, Dhule-424002 (MS) India
**email of corresponding author: rajendraborale@gmail.com*
email of coauthor: sanjaykhodke@gmail.com

Abstract: The present investigation deals with the effect of treatment of Sodium arsenite, on the histochemical components of liver from fish, Mystus bleekeri. This fish was exposed to the sublethal dose of LC_{50} of Sodium arsenite in laboratory conditions for duration desired durations. The histochemical observation revealed that the proteins, lipids and carbohydrates particularly glycogen were depleted. Sodium arsenite toxicity was found time dependent. The fish, Mystus bleekeri is consumed by people; it is essential to know the effect Sodium arsenite on histochemical changes in liver.

Keywords- Histochemistry of liver, Mystus bleekeri, Sodium arsenite, sublethal toxicity

---

Date of Submission: 28-11-2017 Date of acceptance: 09-12-2017

I. INTRODUCTION

The Heavy metals cause serious impairment in metabolic, physiological and structural system of aquatic organism, when present in high concentration [1][2]. Heavy metals accumulated in the tissues of fish may catalyses reactions that generate reactive oxygen species (ROS) which may lead to environmental oxidative stress. Fish are largely being used for the assessment of the quality of aquatic environment and serve as bioindicators of environmental pollution [3][4].

The tremendous increase in the use of heavy metals over the past few decades has inevitably resulted in an increased flux of metallic substances in the aquatic environment [5]. Heavy metals are rapidly discharged into water bodies as wastes and agricultural run-off. Heavy metals are trace metals with a density at least five times that of water, they cannot be metabolized by the organism and hence they are bio-accumulative and inhibit biological processes [6].

An increase of heavy metals toxicity and its bio-accumulation in various tissues of aquatic organisms threatens the biodiversity of ecosystems and health of consumers [7][8]. However, accumulation of metals in fish tissues depends on many factors including environmental factors, type of heavy metals, metal concentration, time of exposure and biological characteristics of fish [9]. In fishes, accumulation of Lead in various tissues and alterations in biochemical and hematological parameters has been reported [10]. Moreover, it has been reported impairment in spermatogenesis, oogenesis, nucleic acids, and protein metabolism in tropical fresh water fishes due to endosulfan [11]. The information on lethal effects of Arsenic on the freshwater fish is scanty. Hence, present work is aimed to investigate the toxicity of Arsenic responses of the freshwater fish *Mystus bleekeri.*

II. MATERIAL AND METHODS

After calculation of the 96-h LC_{50} (16.634ppm) of Sodium arsenite, fish (*Mystus bleekeri*) were exposed to sub-lethal concentration ($1/5^{th}$ of LC_{50}) of Sodium arsenite for 96-h. After exposure, at 24-h, 48-h and 96-h interval, fish was removed from aquarium. The survived fishes were sacrificed and their liver was quickly excised and utilized for histochemical studies from both the control and experimental fishes. After fixation, the tissues were dehydrated through 30 - 100 % alcohol grades and cleared in xylene. Cold and hot impregnations were followed by embedding the tissue in paraffin wax (M. P. 58-60^0 C). Serial sections were cut at 7 µm serial using rotary microtome.

For histochemical detection of protein Mercuric bromophenol blue method [12] was used, for lipid Sudan black-B method [13] and mounted in glycerin jelly and for glycogen, PAS method [14] was employed. All the techniques followed as described by Pearse [15].

III. RESULTS AND DISCUSSION

Mystus bleekeri were exposed to sublethal concentration of Sodium arsenite. The results showed marked histochemical changes, depending on the visualization of variations in intensity of the specific stains.

3.1 Test for proteins:

In the liver cells of normal (control) fish, *Mystus bleekeri* (plate 1, Fig. 01) were characterized by high concentration of proteins are visualized as intensely dark blue coloured granules. The normal hepatocytes demonstrated intense positivity of Bromophenol blue stain, exhibited the presence of a basic and high concentration of total proteins. In the cellular cytoplasm, the Mercury bromophenol blue reaction was either in the form of bluish granules of different size, or in a diffused state, perinuclear or peripheral in position and particularly concentrated adjacent to blood sinusoids. Chromatin bodies and nucleoli exhibited a deep colouration with bromophenol blue.

Total proteins were found to exhibit a noticeable decrease in cytoplasm and nucleus of the liver cells of *Mystus bleekeri*, after exposure to Sodium arsenite. As duration of treatment increased, the diminutions in the protein contents are obvious and the hepatocytes showed cytoplamic vacuolation. The liver of *Mystus bleekeri* after exposure to sublethal concentration 3.33ppm Sodium arsenite at 96-h, showed decreased bromophenol blue reaction as decrease in concentration of protein and their remnants were mainly located at the peripheries of the hepatic cells which showed cytoplamic vacuolation (Plate 1; Fig. 02 to Fig. 04).

3.2 Test for Lipids:

The hepatic cells of the control and treated fishes showed the presence of lipids in the studied tissues. The lipid content of liver tissues of normal (control) fish, *Mystus bleekeri* was appeared to be much more abundant than the treated fish (Plate 1; Fig. 05). It was observed that the individual hepatic cells have designated a variant trend of lipid localization. Some cells appeared more condensed with lipid than other ones.

In the liver cells of normal (control) fish, *Mystus bleekeri*, lipid inclusions were uniformly distributed throughout the cytoplasm, perinuclear and peripheral in position, and particularly accumulated in the cytoplasm adjacent to sinusoids. Most of the hepatic cells exhibited extremely strong diffuse patterns of Sudan black stainability. The sudanophilic granules were coarser and showed a tendency to aggregate into patches either surrounding the nucleus or lying at the periphery of the cells.

It was observed that, lipid inclusions were reduced in liver cells of *Mystus bleekeri* of treated fish with sublethal concentration of Sodium arsenite. Occasional scattered lipid change was observed in some hepatocytes adjacent to the central vein. At 24-h stage (Plate 1 Fig. 06) section showed a slightly reduced Sudan black reactivity and so slight decrease in lipid content, as compared to control. At 48-h stage (Plate 1; Fig. 07), some of the liver cells showed detectable alterations and reduced in the quantity of lipid inclusions. Such depletion of lipid is more pronounced in liver after exposure to sublethal concentration of 3.33ppm Sodium arsenite at 96-h treatment (Plate 1; Fig. 08).

3.3 Test for Glycogen:

Histochemical analysis with Periodic Acid Schiff reaction, glycogen deposits were identified in liver of *Mystus bleekeri*. The PAS preparations of the liver cells of normal (control) fish species, *Mystus bleekeri*, revealed that glycogen was observed in the cytoplasm of the hepatic cells as indicated by large number of reddish or magenta coloured fine granules of different sizes. It is distributed densely in the hepatic cells around the portal area. (Plate 2; Fig. 01)

On the other hand, after exposure of fishes to sublethal concentrations of Sodium arsenite for 24-h, 48-h and 96-h, glycogen content of the liver cells decreased. After 24-h, the hepatocytes showed very slight decrease in the glycogen content in histological section (Plate 2; Fig. 02). After 48-h, the hepatocytes showed slight decrease in the glycogen in histological section (Plate 2; Fig. 03). The decrease was quite evidenced in the amount and stainability. This reduction of glycogen inclusion becomes more pronounced at 96-h. At 96-h, the hepatic cells showed very less amount of glycogen (Plate 2; Fig. 04).

The histochemical tests revealed the localization of chemical products of cellular activity. The altered state of cell constituent can be studied by using histochemical reactions. The intensity of staining can be used for comparing the protein, lipid, glycogen content present the hepatic cells of the normal and treated fishes with Sodium arsenite at different duration. The liver is the primary organ for detoxication of toxicants; particularly heavy metals.

In the present histochemical study, normal (control) fish, *Mystus bleekeri* were characterized by high concentration of proteins, lipids and glycogen in hepatic cells as compare to treated fish with sublethal concentrations of Sodium arsenite. The treated fish showed decreasing trends of proteins, lipids and glycogen in hepatic cells. The depletion of metabolites in this tissue indicates that the whole metabolic pool of the fish gets disturbed or altered under toxic stress. The change in the histochemical contents indicates their rapid utilization to provide excess energy in order to cope with stressful conditions. According to present results on *Mystus*

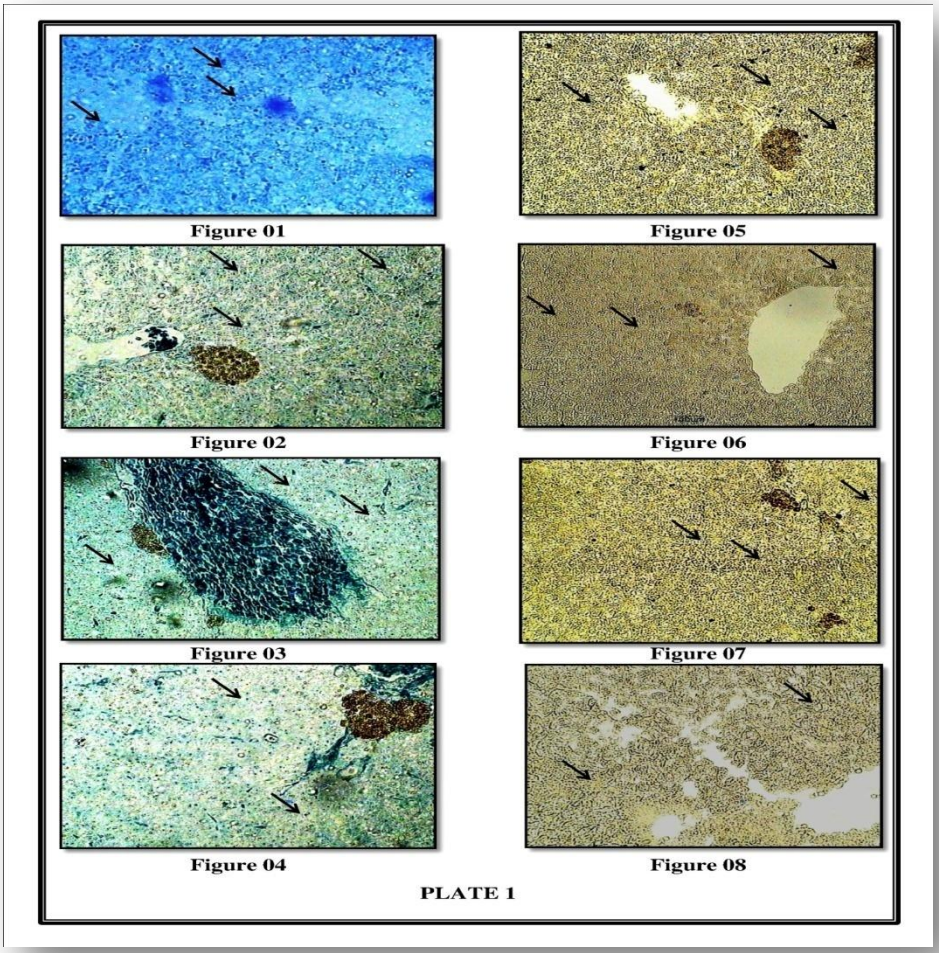
bleekeri, it is suggestive that decreased level of protein, lipid and glycogen in studied tissues may be due to toxic effect of Sodium arsenite or which may impose stress condition.

Similar decreasing pattern was noticed by other workers. Similar histochemical alterations in the fish *Notopterus notopterus* was observed due to the toxic effect of mercuric chloride [16]. The carbohydrate content of liver in the fish species, *Colisa lalia* showed progressive decrease in staining intensity to PAS when treated at sub-lethal and median lethal concentrations of lindane treatment [17]. The effect of chemical effluent on fish species *Mystus vittatus* and found that body constituents like protein, lipid and carbohydrate content of liver, gill, muscles and intestine decreased with increasing concentration of effluent [18].

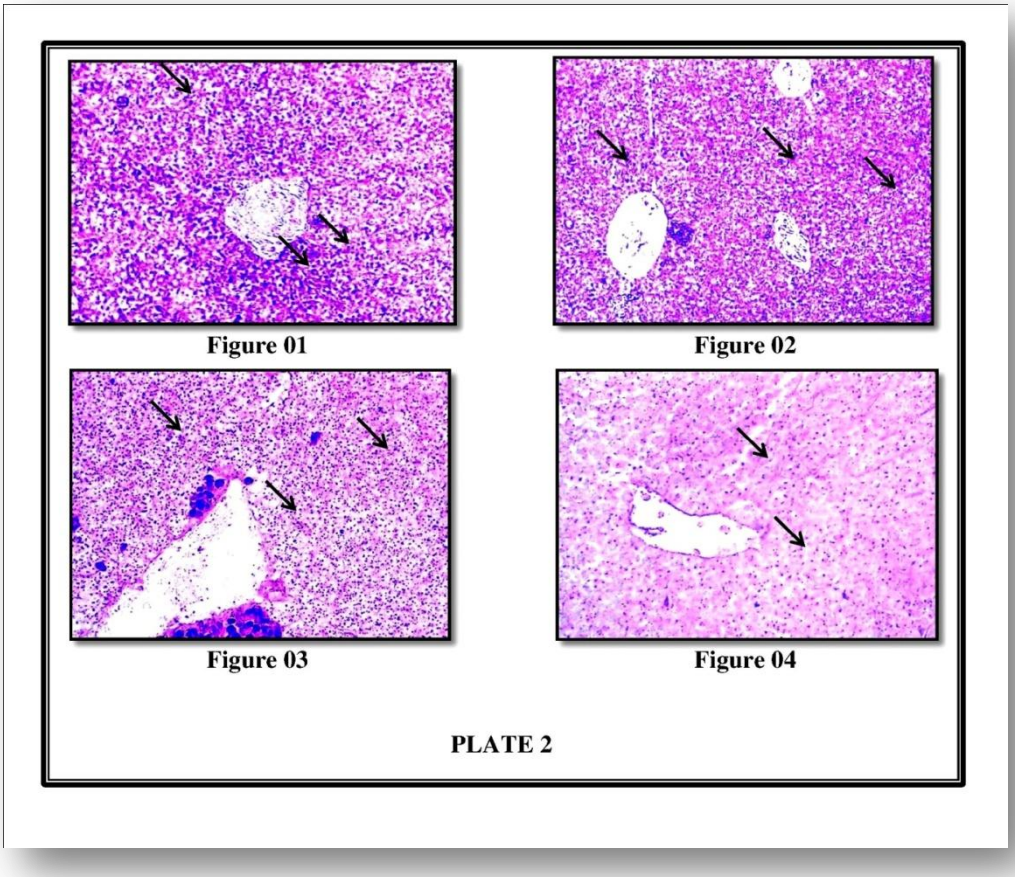
It is reported that the total protein, lipid and glycogen content underwent depletion in the tissue of the tannery effluent treated fish, *Cyprinus carpio* [19]. The study on histochemical changes in the gill of the fish, *Nandus nandus* exposed to sublethal concentration of endosulfan and carbaryl for one month. After long term exposure to both the pesticides, they observed that there is reduction in the carbohydrate contents in all parts of the gills [20]. It also reported that exposing of the fish *Clarias batrachus* to fenvalerate resulted in a highly significant decrease of protein contents of the liver, brain and muscle [21]. The marked reduction in glycogen content of the fenvalerate treated liver cell as compared to the control fish [22].

The study on localization of protein, lipid and glycogen in the liver cells of *Glossogobius giuris* at different period of reproductive cycle after malathion treatment reported significant decrease in protein, lipid and glycogen in the liver of malathion treated fish [23]. The histochemical alterations study in juvenile fish *Oreochromis aureus* treated with sublethal doses of Phenol LC_{50} for 7 days resulted drastic reduction in overall carbohydrates from various tissues [24]. A study showed that paper mill effluent induces reduction in glycogen from liver of freshwater fish, *Rasbora daniconius* [25]. A histochemical study in liver of *Tilapia mossambica* showed that carbohydrate reserves were severely depleted during exposure to $1/10^{th}$ of LC_{50} concentration of sodium fluoride [26].

4. Plate 1: Figures showing localization of Protein and Lipids



4.1 Plate 2: Figures showing localization of Carbohydrate



V. CONCLUSION

The use of histochemical reactions is very realistic approach. The histochemical results from the present study indicate that the Sodium arsenite causes different degrees of injuries to the fish liver. According to present studies on *Mystus bleekeri*, it is suggestive that decreased level of protein, lipid and glycogen, in the liver may be due to toxic effect of Sodium arsenite and or may be due to toxicant imposed stress condition. It is recommended to lessen the use of agriculture chemical fertilizers and pesticides in the fields therefore; the agricultural run-off meet to water reservoirs contains fewer amounts of heavy metals which avoid negative impact on aquatic biota.

ACKNOWLEDGEMENT

The authors are very thankful to the Principal, JET's ZB Patil College, Dhule and Head, Department of Zoology for providing Laboratory facilities, necessary chemicals and instruments to conduct the experiment,.

REFERENCES

[1]. M. Strmac and T. Braunbeck, Isolated hepatocytes of rainbow trout (Oncorhynchus mykiss) as a tool to discriminate between differently contaminated small river systems. Toxicology in vitro, 14(4), 2000, 361-77.

[2]. M. Javed, Relationships among water, sediments and planktons for the uptake and accumulation of heavy metals in the river Ravi. I. J. P. Science, 2, 2003, 326-331

[3]. P.A. Lopes, T. Pinheiro Santos and A.M. Viegas Crespo, Response of antioxidant enzymes in freshwater fish population to inorganic pollutants exposure. Sci. Total Environment, 280, 2001, 153-163.

[4]. C. Dautremepuits, S. Betoulle, S. Paris- Palacios and G. Vernet, Immunol-related perturbations induced by copper and chiston in carp Cyprinus carpio. Arch Environ. Contam. Toxicology, 47, 2004, 370-378.

[5]. H. Yang and N.L. Rose, Distribution of Hg in the lake sediments across the UK. Sci.Total Environment, 304, 2003, 391-404.

[6]. S. Das and A. Gupta, Acute toxicity studies on Indian flying barb, Esomus Danricus (Hamilton-Buchanan), in relation to exposure of heavy metals, cadmium and copper. J. of Environ. Res. and Development, 4(3), 2010, 705-712.

[7]. R. Vinodhini and M. Narayanan, Effect of heavy metals induced toxicity on metabolic biomarkers in common carp. Internat. J. of Environ. Sci. and Technology, (1): 2008, 192-200.
[8]. K.R. George, N.A. Malini, A. Rajan and R. Deepa, Enzymatic changes in the kidney and brain of freshwater murrel, Channa striatus (Bloch) on short term exposure to sub-lethal concentration of Lead nitrate. Indian Journal of Fishery, 58(4), 2011, 91-94.
[9]. B. Jezierska and M. Witeska, The metal uptake and accumulation in fish living in polluted waters. In: Twardowska I., Allen H.E., Häggblom M.M., Stefaniak S. (eds) Soil and Water Pollution Monitoring, Protection and Remediation. NATO Science Series, vol 69, 2006, 107-114, Springer, Dordrecht.
[10]. B. Ates, I. Orun, Z.S. Talas, G. Durmaz and I.Yilmaz, Effects of sodium selenite on some biochemical and hematological parameters of rainbow trout (Oncorhynchus mykiss Walbaum, 1792) exposed to $Pb2^{+}$ and $Cu2^{+}$. Fish Physiol. Biochemistry,, 34, 2008, 53-59.
[11]. L. Shukla, and A. K. Pandey, Effect of endosulfan hypothalamo hypophysical complex and fish reproductive physiology. Bull. Environ. Contam. Toxicol., 36, 1986, 122-131.
[12]. D.M. Chapman, Dichromatism of bromphenol blue, with an improvement in the mercuric bromphenol blue technic for protein. Stain Technol. Jan; 50(1), 1975, 25-30.
[13]. J.R. Baker, The histochemical recognition of lipine. Quart. J. Micr. Science, 87, 1946, 409-470.
[14]. R.D. Hotchkiss, A micro chemical creation resulting in the staining of polysaccharide structure in fixed tissue preparations. Arch. Biochemistry,16, 1948, 131.
[15]. A.G.E. Pearse, Histochemistry the practical and applied 2nd Ed., 1961, J. and A. Churchill Ltd; London.
[16]. S.R. Verma and Chand, Toxicity effects of mercuric chloride on freshwater enzymes of carbohydrate metabolism of Notopterus notopterus. Indian J. Environ. Health, 28, 1986, 1-7.
[17]. P.R. Karpaganapthy, P. Dharamani, C.N. Rajamanikam, A. Prakasam and K.V. Rengarajan, Histochemical studies on the effects of lindane in the anabantid fish, Colisa lalia (Hamilton Buchanan). Proc. Second National symp. Ecotoxicology, 1988, 188-190.
[18]. S. Palanichamy, S. Arunachalam Ali Mohammed and P. Baskaran, Effect of chemical effluent on feeding energetic and body composition in the fresh water catfish Mystus vittatus, 1989, Proceed second Asian Fisheries Forum, Japan.
[19]. S.J.K. Naik, VV Devi and S Piska, Toxicity of tannery effluent on carbohydrate, protein, lipid constituents in the selected tissue of Cyprinus carpio (Linnaeus). J. Aqua. Biology, 19(1), 2004,177-181.
[20]. S. Khare and S. Singh, Histochemical alterations in the gill of the fish Nandus
[21]. nandus due to pesticidal exposure. Nature Env. Polln. Technology, 3(4), 2004, 535 -538.
[22]. G. Tripathi and P. Verma, Fenvalerate-induced changes in a catfish, Clarias batrachus: metabolic enzymes, RNA and protein. Comp. Biochem. Physiology, 138(1), 2004, 75-79.
[23]. S.A. Sakr and S.M. Jamal Al Laial, Fenvalerate induced histopathological and
[24]. histochemical changes in the liver of the catfish, Clarias gariepinus. J. App. Science, 1(3), 2005, 263-267.
[25]. UD Anandhi and PS Murthy, Chemical stress on the liver of Glossogobius giuris (Ham) an overview. Adv. Env. Technology, 2006, 79-96.
[26]. S. Sabry, H. El-serafy Nassr-Alla, Abdel-Hameid and A. Amal El-Daly, Histological and histochemical alterations induced by phenol exposure in, Oreochromis aureus (Steindachner, 1864) juveniles. Egypt. J. Aquat. Biol. and fish, 13(2), 2009, 151-172.
[27]. T.S. Pathan, P.B. Thete, S.E. Shinde, D.L. Sonawane and Y.K. Khillare, Histochemical changes in the liver of freshwater fish, Rasbora daniconius, exposed to paper mill effluent. Emir. J. Food Agriculture, 21(2), 2009, 71-78.
[28]. M.B. Bagale, K.R. Rao, S.S. Kshirsagar and N.V. Shah, Sodium fluoride induced histochemical changes in the liver of freshwater fish, Tilapia mossambica (Oreochromis mossambicus). The Bioscan, 9(3), 2015, 1089-1092.

Borale Rajendra P "Sodium arsenite induced histochemical changes in the liver of fish Mystus bleekeri (Day)." IOSR Journal of Engineering (IOSRJEN), vol. 07, no. 12, 2017, pp. 05-09.