Toxicology Reports 3 (2016) 148–152

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Arsenic induced hematological and biochemical responses in nutritionally important catfish *Clarias batrachus* (L.)

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ARTICLE INFO

Article history:
Received 18 August 2015
Received in revised form
20 December 2015
Accepted 4 January 2016
Available online 8 January 2016

Keywords:
Clarias batrachus
Cholesterol
Hematological indices
Sodium arsenite
Toxicity

ABSTRACT

The impact of sublethal toxicity of sodium arsenite on hematological and certain biochemical parameters of the fresh water catfish *Clarias batrachus* has been analyzed following exposure of sublethal concentration (1 mg/L; 5% of LC_{50} value) of sodium arsenite for 10, 30, 45, and 60 days. Arsenic bioaccumulation in the blood tissue of the fish increased progressively with increased period of exposure. The values of total erythrocyte count (TECs), total leucocytes count (TLCs), hemoglobin concentration, and packed cell volume (PCV) $1.40 \pm 0.03 \times 10^6/mm^3$, $174.83 \pm 2.74 \times 10^3/mm^3$, 5.01 ± 0.26 g/100 ml, 25.00 ± 1.06 were observed respectively at the end of 60 days of exposure. The results of hematological indices were found to be 179.23 ± 8.81fl/cell for mean corpuscular volume (MCV), 35.92 ± 1.89 pg/cell for mean corpuscular hemoglobin (MCH) and 20.17 ± 1.12 g/dl for mean corpuscular hemoglobin concentration (MCHC). The present findings are clearly indicating severe fish anemia due to the arsenic salt exposure. The continued arsenic toxicity results in decreased serum protein concentration that might be a cause for the loss of weight as well as weakness in the fish.

1. Introduction

Arsenic is a widespread chemical in the aquatic environment due to both geogenic processes as well as anthropogenic disturbances [9,34]. Increased concentrations of arsenic in groundwater have been reported from several countries, including India, Bangladesh, China, Japan, Nepal, Taiwan, Vietnam and some parts of the United States [18,4]. Recently the wetlands of neighboring districts of Varanasi (Ghazipur, Balia, *etc.*) were found to be extensively contaminated with arsenic [56]. Elevated concentration of arsenic has raised great concern from both environmental and human health perspectives. Arsenic has been identified as one of the most alarming chemicals [7]. Its trivalent salt (sodium arsenite) is more toxic than other forms. Hence, sodium arsenite was preferred as the test toxic component. The aim of this work is to illustrate the arsenic induced impairments in fish, which is an important source of all essential amino acids.

In fish, blood shows the early impact of arsenic toxicity as it enters the blood predominantly through extensive gill surface area where the barrier between the blood and the metal salt is very thin [38] as well as through buccal cavity. Other metals (mercury, chromium, and nickel) and synthetic pyrethroids such as azodrin, cypermethrin, fenvalerate and mancozeb also exert acute toxicity on blood in different fish species [14,16,6]. For the last several decades, fishes have been used widely as a model organism to assess the impact of contaminated water. Very few workers like [56,51]; have worked on arsenic toxicity in fish. Hardy nature of *Clarias batrachus* makes it an excellent bioindicator animal model for toxicological investigations. Blood parameters have been widely employed as pathophysiological indicators to diagnose the structural and functional status of fishes exposed to a variety of toxicants [1]. Hematological indices like hemoglobin (Hb), red blood corpuscles (RBCs), packed cell volume (PCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) have regularly been used to assess the oxygen carrying capacity of the blood as well as an indicator of metal pollution in aquatic environment [54]. Analysis of serum biochemical parameters especially useful to identify target organs of toxicity as well as the general health status of animals, and is advocated to provide early signs of critical modifications in stressed organisms [31,35]. Besides, biochemical investigations were used to illustrate the toxicity on different tissue systems. Hence, this investigation is aimed at studying the changes in hematological as well as biochemical status of the blood tissue of arsenic exposed *C. batrachus.*

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http://dx.doi.org/10.1016/j.toxrep.2016.01.001

Table 1
Hematological parameters (mean ± SEM) of *C. batrachus* exposed to sublethal concentration of sodium arsenite (1 mg/L water) at different exposure duration (10–60 days); in parentheses are percent change of value compared to control group.

	Hemoglobin (g/dl)	Total RBCs ($\times 10^6$/mm^3)	PCV (Ht)	MCV (fl/cell)	MCH (pg/cell)	MCHC (g/dl)	TLCs ($\times 10^3$/mm^3)
Unexposed (Control)	10.80 ± 0.426	2.34 ± 0.03	47.33 ± 1.56	153.12 ± 9.62	46.20 ± 2.08	30.76 ± 2.29	107.18 ± 2.90
10 days	7.98 ± 0.48** (−26.11)	2.19 ± 0.03* (−6.41)	38.33 ± 1.14** (−19.01)	142.61 ± 8.07^{NS} (−6.86)	36.46 ± 2.49* (−21.08)	25.62 ± 1.14^{NS} (−16.71)	129.00 ± 1.57*** (+20.35)
30 days	7.07 ± 0.24*** (−34.53)	2.11 ± 0.020*** (−9.82)	36.66 ± 1.56*** (−22.54)	145.77 ± 10.5^{NS} (−4.80)	33.53 ± 1.26** (−27.42)	23.69 ± 2.10* (−22.98)	150.41 ± 1.29*** (+40.33)
45 days	6.64 ± 0.12*** (−38.52)	1.84 ± 0.019*** (−21.36)	34.00 ± 1.46*** (−28.16)	167.80 ± 7.2^{NS} (+9.58)	36.15 ± 1.05** (−21.75)	21.71 ± 0.99** (−29.42)	159.83 ± 2.37*** (+49.12)
60 days	5.01 ± 0.26*** (−53.61)	1.40 ± 0.03*** (−40.17)	25.00 ± 1.06*** (−47.18)	179.23 ± 8.81^{NS} (+17.05)	35.92 ± 1.89** (−22.25)	20.17 ± 1.12** (−34.42)	174.83 ± 2.74*** (+63.12)

The statistical difference between the group means compared to control group is indicated as follows: * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$) and NS-non significant.
Abbreviations:Hb, hemoglobin; RBCs, red blood cells; Ht, haematocrit; PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular haemoglobin concentration; TLCs, total leucocyte counts.

2. Materials and methods

Fresh water catfish *C. batrachus* from a single population (body length 15 ± 1 cm and body weight 45 ± 5 g), were collected from the local fish market at Varanasi. They were maintained in large plastic containers bearing 20 Liters of tap water (dissolved O_2 6.3 mgL^{-1}, pH 7.2, hardness 23.2 mgL^{-1}, and temperature 28 ± 3 °C) for 21 days for acclimation. Freshly minced goat liver was used to feed *ad libitum* after every 48 h. Water was renewed after every 24 h. In this investigation, we have exposed the fish to sublethal concentration of sodium arsenite. Although, we have previously measured the 96 h median lethal concentration (96 h LC_{50}) of sodium arsenite (Batch No G270707 Loba Chemie Pvt. Ltd., Mumbai, minimum assay 98.5–102% pure) by standard method [11]. Hence, for this investigation we have taken twenty groups of 10 fish which were exposed separately to sublethal concentration (1 mg/L; 5% of 96 h LC_{50} value) of sodium arsenite in large plastic aquaria containing 10 L of the arsenic solution prepared in the tap water. Another twenty groups of 10 fish taken as controls which were retained in 10 L of plain tap water (without having the arsenic salt) under identical laboratory conditions. For hematological analyses, fish (three fish from each three experimental as well as control aquaria) were cold anesthetized and sacrificed by spinal dislocation after the completion of 10, 30, 45, and 60 days of exposure. Blood samples were collected from caudal vein of these fish. Hematological values were measured following standard methods. Hemoglobin (Hb) was estimated by Sahli's acid haematin method as described by Darmady and Davenport [23]. Red blood cells (RBC) and white blood cells (WBC) were counted by Neubauer's improved haemocytometer using Hyem's and Turk's solution as a diluting fluid respectively described by Darmady and Davenport [23]. The microhaematocrit method of Snieszko [25] was used to determine the hematocrit/packed cell volume (PCV). The derived hematological indices of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated using standard formulae [22,37]. MCV was calculated in femtoliters = PCV/RBC × 10; MCH was calculated in picograms = Hb/RBC × 10; and MCHC = (Hb in 100 mg blood/PCV) × 100.

Serum glucose was estimated by the method of Cooper and McDaniel [21] and serum protein was detected by the method of Lowry et al. [40], while both total serum cholesterol and HDL were estimated by the method of Nadar et al. [45]. The data in this paper have been presented with mean ± mean standard error (SEM) and the statistical significance of differences between control and experimental group was evaluated by two tailed student's *t*-test using the SPSS program, version 12. The criterion for significance was set at $p < 0.05$, $p < 0.01$ and $p < 0.001$.

3. Results and discussion

The toxicity of arsenic induced anemia in *C. batrachus* is due to progressive decrease in its hemoglobin content (55.61% from control) as presented in Table 1. According to Blaxhall and Daisley [12] the depletion of hemoglobin content is an excellent gauge of anemic condition of the fish. Oladimeji et al. [46] also reported significant decline in hemoglobin content of rainbow trout exposed to different concentration of arsenic. Another metal salt mercuric chloride caused similar significant decreases in the hemoglobin content in the *C. batrachus*. Devi and Banerjee [29,30] observed decreased hemoglobin content in the blood of *Channa striata* following exposure to sublethal toxicity of aluminum sulfate and lead nitrate. However, they noticed periodic fluctuations of the hemoglobin content at different stages of exposure to both the contaminants. Shah and Altindag [54] noticed decrease in hemoglobin, RBC count and PCV values in *Tinca tinca* exposed to mercury and lead salts. Decrease in RBC count, hemoglobin, and PCV values were also noticed in *Nile tilapia* exposed to the pesticide edifenphos. Decreased rate of production of red blood cells or an increased loss of these cells in arsenic exposed *C. batrachus* might be the main reason for hemoglobin depletion. According to Reddy and Bashamohideen [50], the significant decrease in hemoglobin concentration of fishes under toxic stress could be either due to increased rate of destruction of hemoglobin or due to decrease rate of synthesis of hemoglobin. The other reason for progressive reduction in hemoglobin concentration might be the consequences of depression/exhaustion of hemopoitic potential of the fish [53,26,27]. The third reason for the decreased hemoglobin content might be due to suppression of hemopoitic activity of the kidney in addition to the increased removal of dysfunctional RBCs following exposure. Chen et al. [17] found that Tilapia potentially regulate the concentration of metal in the tissue with time by combining the process of absorption, excretion, detoxification and storage. Kumar and Banerjee [11] also found that the amount of arsenic uptake is organ specific. According to Gill and Epple [32] the reasons for anemia might be impaired erythropoisis caused by the direct effect of metal on hematopoietic centers (kidney/spleen), accelerated erythroclasia due to altered membrane permeability and/or increased mechanical fragility, and defective iron metabolism or impaired intestinal uptake of iron due to mucosal lesions. Progressive decrease in the RBCs might be one of the main causes of anemia. The decrease in RBCs density and hemoglobin content resulted in diminished oxygen supply. According to Buckley et al. [13], prolonged reduction in hemoglobin content could be deleterious to the oxygen transport and any blood dyscrasia and degeneration of RBCs could be endorsed as pathological condition in fishes exposed to toxicant. Other toxicants (ammonium sulfate, cypermethrin) including heavy metals

Table 2
Biochemical parameters (mean ± SEM) of *C. batrachus* exposed to sublethal concentration of sodium arsenite (1 mg/L water) at different exposure duration (10–60 days).

	Arsenicconcentration in blood (μg/g) $^{\psi}$	Proteins(mg/dl)	Glucose(mg/dl)	HDL (mg/dl)	Cholesterol(mg/dl)
Unexposed(Control)	<DL	3.80 ± 0.57	20.96 ± 0.76	46.37 ± 3.05	235.94 ± 2.86
10 days	1.74 ± 0.03	4.36 ± 0.14^{NS}	26.56 ± 1.07^{NS}	35.52 ± 4.36*	246.96 ± 2.03**
30 days	4.05 ± 0.14***	3.26 ± 0.51^{NS}	34.63± 0.66**	34.05 ± 4.29**	261.20 ± 2.79***
45 days	5.48 ± 0.26***	2.78 ± 0.53**	41.06 ± 0.86***	32.81 ± 2.19***	224.98 ± 6.88*
60 days	6.07 ± 0.04***	1.52 ± 0.39**	48.5 ± 1.36***	32.33 ± 1.88***	209.52 ± 5.01***

The statistical difference between the group means compared to control group is indicated as follows: * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$) and NS—non significant. Symbol: $^{\psi}$ [11], <DL—below detection limit.

(zinc, lead, copper, mercury) also induces decreased erythrocyte counts in several fishes [24,59,60,28,29,41,58]. The reduction in RBCs count was also dose dependant [41]. Packed cell volume (PCV) of control fish was 43.33 ± 1.56. It decreased progressively throughout the period of exposure. The progressive decrease in PCV values is due to the decreased number of RBCs (Table 1). Other metallic compounds (mercuric chloride and aluminum chloride) also caused significant decrease of PCV value in fish [41,3]. Atamanalp and Yanik [5] and Patnayak and Patra [36] also observed decrease PCV following exposure to various pesticides. PCV value also decreased in *C. striata* exposed to ammonia and lead [28,29]. Coles [20] suggested that hematological indices like MCV, MCH and MCHC are important indicators in diagnosis of anemia in most animals. The MCV is an indicator of status or size of the red blood cell and reflects the normal or abnormal cell division during erythropoisis. MCV value of *C. batrachus* decreased (153.12 ± 9.62 in experimental was compare to 145.77 ± 10.5 control group) after 30 days of exposure due to decreased number of RBCs causing anemia (Table 1). However, MCV value found significantly increased after 45 and 60 days of exposure as compare to previous exposed group. Sinha et al. [57] also observed increase level of MCV and Erythrocyte sedimentation rate in *Labeo rohita* due to copper intoxication and suggested that the anaemia was of macrocytic type. Devi and Banerjee [30] also found increased level of MCV value in *C. straiata* following exposure of ammonium sulfate. Following exposure the normal MCH value decreased substantially but fluctuated in narrow ranges at different stages of exposure (Table 1). Significant decrease in MCH value was also been reported in ammonia and toxic metal [6,28,29] exposed fishes indicating micro cystic anemia. Decrease in MCH and MCV level indicate hypochromic microcytic anemia [55]. MCHC measurement is a diagnostic tool to assess the amount of RBC swelling (decreased MCHC) or shrinkage (increased MCHC) [43]. The MCHC value of exposed *C. batrachus* decreased steadily and became 20.17 ± 1.12 g/dl after 60 days of exposure (from 30.76 ± 2.29 g/dl in control). This shows arsenic causes swelling of the RBCs. Devi and Banerjee [29] also reported decreased MCHC value in *C. striata* following exposure to ammonia. The same author Devi and Banerjee [28] observed decreased MCHC value in *C. striata* after 60 days of lead exposure. However, the MCHC in this case showed periodic fluctuations at earlier stages of exposure. Alwan et al. [3] observed high MCHC as well as MCH value following exposure to aluminum indicating large size RBC containing less hemoglobin content. The total leucocytes count in arsenic treated fish increased steadily (from 107.18 ± 2.90 × 103/mm^3 in control to 174.83 ± 2.74 × 103/mm^3 after 60 days of exposure) (Table 1). The increase in total leucocytes count may be attributed to increase immune reaction perhaps to protect the fish already damaged by the arsenic stress. Leucocytes play a major role in the defense mechanism of the fish [49]. Decrease in RBC count and Hb concentration along with increased leucocytes count were also noticed when the same fish was exposed to different concentration of $HgCl_2$ [41]. Ayoola [8] also observed leucocyte counts in the fish fed with compounded feed and suggested that the increase was due to enhanced production of leucocytes in the hemopoitic tissue of the kidney and spleen. In the same fish *C. batrachus* Maheswaran et al. [41] also observed increased leucocytes count due to stimulation of immune system caused by tissue damaged following exposure to mercuric chloride. Several other toxic elements induced the leucocyte counts in fishes [2,39,54]. According to Wedemeyer and Wood [61] the primary consequence of changes in the leucocytes in stressed fish is suppression of the immune system and increased susceptibility to disease. Gill and Pant [33] found increased leucocytes count due to stimulation of the immune system rendered by injury of tissue damaged. On the other hand Devi and Banerjee [28] and Devi and Banerjee [29] observed decreased leucocytes count in *C. striata* exposed to ammonium and lead salts.

The serum glucose content of unexposed control fish was 20.96 ± 0.76 g/dl. In the exposed fish the serum glucose level increased progressively throughout the period of exposure (Table 2). The serum glucose level of the exposed fish increased significantly after 10 days (Fig. 1a) of exposure. The increased level of glucose might be due to liberation of carbohydrates following breakdown of vital macromolecules like proteins and high density lipids from different organ systems, resulting in progressive decrease of lipid and protein concentrations. Arsenic also disturbs the glucose metabolism by uncoupling of oxidation and phosphorylation [44] causing excessive availability of unutilized glucose molecules in the tissue. These additional molecules of glucose might later have got converted into glycogen or fat causing increased glycogen/lipoidal concentration. Increased level of serum glucose has also been reported in fishes exposed to cadmium [15,48,19] and several other stressor including heavy metals [14]. The serum protein concentration of unexposed control fish was 3.80 ± 0.577 g/dl. In the experimental fish group with exception of 10 days of exposure the serum protein progressively decreased throughout the period of exposure (Table 2). On the other hand, serum protein level decreases significantly after 30 days of exposure (2.78 ± 0.53 in experimental compare to 3.80 ± 0.57 mg/dl in control group; Fig. 1b) indicating regular depletion of these macromolecules. The lowering of protein concentration was perhaps accompanied by the glucose increase, to meet the high energy demand necessary to struggle with the arsenic stress. Kumar and Banerjee [38] also noticed depletion of glycogen and proteins from the hepatic and muscular tissue of *C. batrachus* following arsenic exposure. They also correlated the decrease with release of additional amount of carbohydrate to meet the toxic stress of arsenic. Similar decrease in the glycogen level in the muscles and liver of certain fishes exposed to heavy metals were also observed [19].

The serum cholesterol and high density lipoprotein contents of unexposed control fish were 235.94 ± 2.86 and 46.37 ± 3.05 mg/dl, respectively (Table 2). In the exposed fish serum HDL decreased progressively throughout the period of exposure (Fig. 1c). The serum cholesterol level decreased after 45 days onwards, at shorter exposure time the serum cholesterol level increased, what was highly significant after 60 days of exposure (Fig. 1d).

Various other metals also caused decrease in level of serum cholesterol in different fish species [14,62,58,47]. The cholesterol is known to be essential structural component of membranes and also precursors of all steroid hormones [44]. The cholesterol level induced by heavy metals might be due to liver failure which sub-

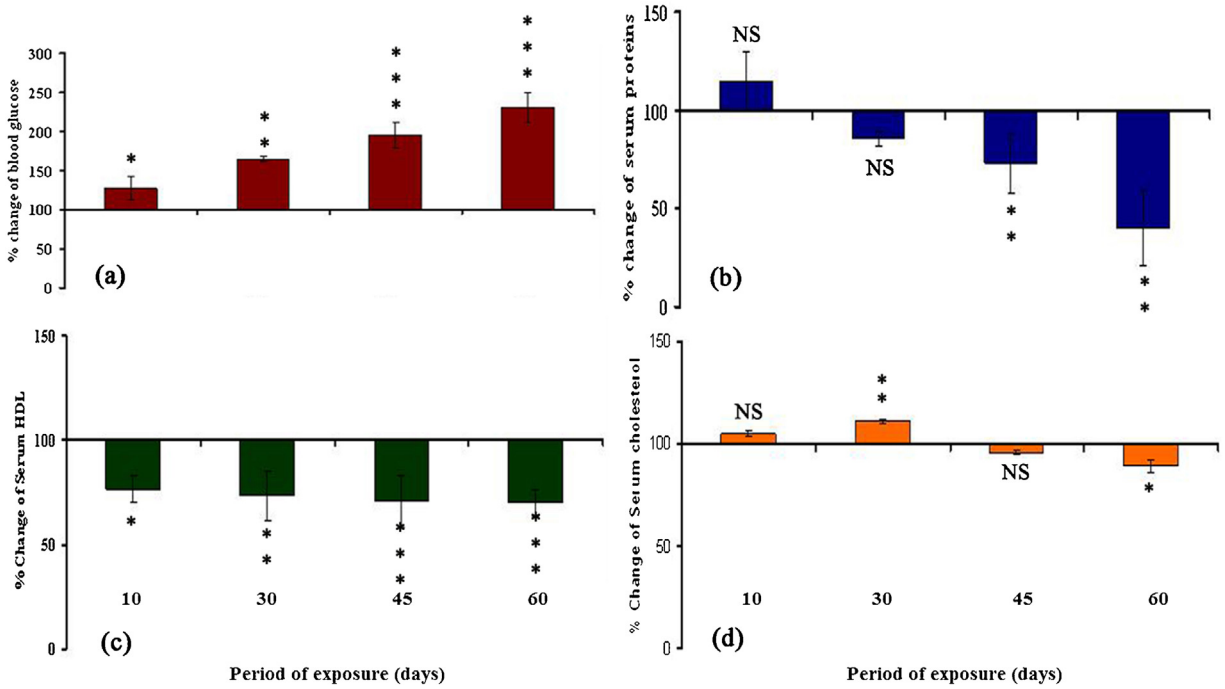

Fig. 1. Illustration of percentage change of macromolecules (a)—glucose, (b)—protein (c)—HDL, (d)—cholesterol in the blood tissue of *C. batrachus* at different periods of exposure to 1 mg/L of sodium arsenite. Value of untreated control is taken as 100%. The statistical difference between the group means compared to control group is indicated as follows: * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$).

sequently leads to elevate the concentration into the serum. Heavy metals damaged the membranes hence very often increase level of cholesterol is taken as indicators of environmental stress. Following Santra et al. [52] they concluded that this observation was due to disturbed lipid metabolism rendered by arsenic stress. Insecticide exposure also increases lipid concentration in the fish which perhaps due to increased range of acetyl coenzyme-A to acetoacetate units for lipogenesis [10]. These authors inferred that increased oxidative stress may be due to mitochondrial damage within the hepatocytes that in turn causes decreased mitochondrial oxidation of fatty acids. These fatty acids are shunted towards esterification pathways resulting in accumulation of triglycerides within the hepatocytes which seems be true in our study but also in the study of Kumar and Banerjee [38]. High density lipoprotein (HDL) level of the serum of control *C. batrachus* was 46.37 ± 3.05 mg/dl, that is quite high compared to the minimum level of HDL (35 mg/dl) required for human health. *C. batrachus* which is a very important fish both nutritionally as well as medicinally and has immense food value shows diminution in nutritional value as arsenic exposure causes progressive decrease in HDL level (Fig. 1c). Metwally et al. [42] also reported decreased level of HDL following exposure to other toxic elements copper and zinc. The content of arsenic in blood of unexposed control fish was below the detection limit (Table 2). However, the level of arsenic concentration increased significantly throughout the period of exposure.

4. Conclusions

The present study thus confirmed that hematological parameters are very sensitive parameters for monitoring toxic responses of the fish following exposure to sodium arsenite. Arsenic intoxication reveals the anemic condition in fish species. We assume that alterations in hematological indices may be a defensive mechanism against arsenic toxicity through stimulation of leucopoiesis.

Acknowledgment

The senior author is thankful to the UGC Government of India New Delhi for providing Senior Research Fellowship.

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