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Research article

Evaluation of Serum Biochemical Parameters of *Cirrhinus mrigala* Under Acute Exposure of Arsenic

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Abstract

Arsenic, a toxicant of fresh water habitat have damaging effects in fish. The present study was carried out to evaluate the lethal toxicity of As_2O_3 to *Cirrhinus mrigala* at different exposure durations comprised 24-hour, 48-hour, 72-hour and 96-hour along with the analysis of different serum biochemical parameters. Different constituents of serum such as sodium, potassium, Chlorine, urea, protein, glucose, albumin, alanine aminotransferase and aspartate aminotransferase were analyzed in control and arsenic exposed groups. The results depicted prominent variations among all parameters of serum under acute exposure of arsenic trioxide. The levels of Na^+, Cl^- and albumin were recorded lower at all exposure durations of arsenic as compared to the control group. However, the levels of K^+, total protein, urea, total glucose, AST and ALT were recorded higher in all arsenic exposed fish groups in comparison to control group. The results indicated that arsenic trioxide has an adverse effect on serum biochemical parameters and these serum constituents could be used as bioindicators to assess the toxicity caused by As_2O_3 and thus they help in assessing the health status of fish.

Keywords: Acute, arsenic, *Cirrhinus mrigala*, blood, serum

Graphical Abstract
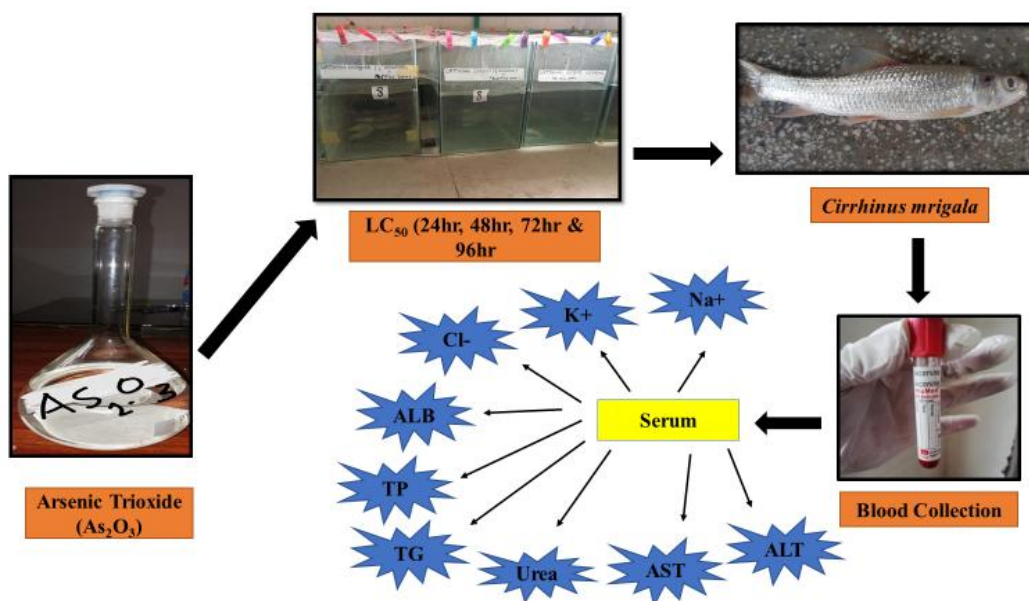
1. Introduction

The presence of diversity of Pollutants (organic, inorganic, nutrients and agricultural, suspended solid, radioactive, pathogens) in freshwater habitat has become an important matter of concern in Pakistan [1, 50]. Due to development in industries and migration to the cities, quantity and the level of contamination has been increasing for about last 10 years [2]. Heavy metals in the fresh water either in highly or in less lethal form, are the extra source of problem (affecting the individual growth rates, physiological functions, mortality and reproduction in fish) in fresh water habitats [3]. In natural water resources arsenic is a major problem all over the universe which causes severe problems for all the living beings because it is very strong toxicant in the natural habitats [4, 5]. In groundwater, arsenic contamination has affected almost 108 countries. About 32 countries of Asia and 31 countries of Europe, followed by 20, 11, 9 and 4 regions of Africa, North America, South America and Australia respectively. Southeast Asian countries including Bangladesh, India, Pakistan, China, Nepal, Vietnam, Burma, Thailand and Cambodia, are the most affected. [47].

Arsenic is a naturally occurring ubiquitous element in aquatic water bodies due to many natural and human activities [3, 6, 7, 4]. In the environment, arsenic mostly comes from volcanic eruptions, weathering of arsenic containing rocks and as a result of some biological activities. Moreover, widespread applications of arsenic containing wood preservative, pesticides and herbicides over a long period of time are also an important source of arsenic [48]. Arsenic is ranked as one of the most dangerous chemical in the natural habitats as it exists in different chemical forms. Its tendency of oxidation and adversity determines the level of toxicity [8]. Among the different arsenic containing molecules, arsenic trioxide is abundantly utilized to produce many farming chemicals and carbon containing chemical. [9]. Natural sources of arsenic are erosion of rocks, emission of hot lava and different activities by living organisms such as digging of soil to extract minerals, use of chemicals to kill pests and chemicals to protect the woods from pests which caused the contamination of soil [7, 10].

Fish are effective bio indicators of arsenic toxicity because they face the toxicity of arsenic via the gills during respiration in freshwater habitat [11-13]. Being at top of the food chain in aquatic ecosystem, fish accumulate the significant amount of Cd from the aquatic habitat [14]. Fishes are continuously exposed to the stress environment that causes fluctuation in their biochemical and hematological parameters therefore these are widely used to monitor the stress conditions [15].

Under the heavy metals exposure different serum biochemical parameter (such as ALT and AST) are used as stress indicator [16, 17]. Bio indicators are utilized to assess the quality of environment and health of organisms [18-20]. Blood is a good indicator of presence of toxicity in surroundings because blood indices are sensitive and fast reacting biomarkers of several environmental effects, including water pollution with toxic agents [21, 49]. Evaluation of serum biochemical parameters indicates the impact of toxicants on these biochemical parameters because fish give quick response to the toxicants depending on the level and kind of pollutant [22]. Evaluation of biochemical constituent also helps to assess the mode of action of toxicants and assessment of freshwater habitats. Moreover, the degree of toxicity imposed by chronic exposure of metals is also indicated by the measurement of different enzymes of serum such as ALT and AST levels [23-25]. The very crucial biomolecules that control the different anabolic and catabolic activities are enzymes thus even a trivial change in activities of enzymes can cause the death of an organism [26].

To monitor the physical status of animal species (Aquatic animals i.e. fish) in concern serum biomolecules like ALT and AST enzymes are categorized as most vital indictor in a biological system [27, 28]. The most abundant enzymes like AST and ALT are very crucial in anabolism and catabolism of protein [28, 29]. Stress causing pollutants such as metal exposures, in environment cause variations in the serum biochemical parameters (Total protein, Glucose, Blood urea nitrogen, Creatinine, Cholesterol) [30-32]. Therefore, a research was conducted to assess arsenic toxicity by using serum biochemical parameters as bio indicators.

2. Materials and Methods

2.1. Fish sampling and experimental layout

A total of 60 Fingerlings of *Cirrhinus mrigala* were brought from fish seed hatchery, Begowala and experiment was conducted in zoology lab of Govt. College Women University Sialkot. Fish were acclimatized for 24h under laboratory conditions. Physico-chemical parameters of water i.e. temperature (28 ºC), pH (7.00), water hardness (300mg/L), dissolved oxygen (5.7 mg/L) concentrations, free CO_2 (10.50 mg/L), ammonia (0.15 mg/L), calcium (24.30 mg/L) and magnesium (59.81 mg/L) were determined by using standard methods [33]. To maintain the quality of water on daily basis while the first three parameters were kept constant. Twenty fingerlings were divided into two groups i.e. control and treatmental group. Arsenic trioxide (As_2O_3) of molecular weight 197.84 g/mol was taken for the experiment. The arsenic stock solution was serially diluted to make required concentration of metal ion solution. Fish under the acute exposure of As_2O_3 were not fed while control group was not treated with arsenic. Entire experiment was conducted in three replicates.

2.2. Measurement of arsenic acute toxicity

LC_{50} was obtained at 30 ppm and death rate of fish was determined after 24 hr, 48 hr, 72 hr and 96 hr. No fish died in the control groups while dead fish from arsenic exposed groups (ten) were removed immediately. Obtained data during the whole research was statistically analyzes to compare the different samples and to find out the significance of the obtained data. Moreover, relationship of dose with exposure duration was also assessed statistically.

2.3. Probit analysis

Mortality data obtained after acute toxicity exposure (24 hr, 48 hr, 72 hr, and 96 hr) was evaluated by applying probit test, to analyze relationship between dose (arsenic) and its effect on biochemical parameters (response) with 95% confidence interval [34].

2.4. Determination of serum biochemical parameters

From each aquarium blood samples of three fish without anticoagulant were collected for determination of concentration of different serum biochemical parameters such as Na^+ (mmol/L), K^+ (mmol/L), Cl^- (mmol/L), Alb (g/dL), Tp (g/dL), Urea (mg/dL), Tg (mg/dL), Ast (U/L) and Alt (U/L) were measured for experimental groups (after 24, 48, 72 and 96 hours of arsenic exposure) as well as for control group by using standard methods and commercially available kits were used (Table: 1).

2.5. Statistical analysis

The probit analysis by Minitab software was applied to evaluate mortality data. Probit analysis determined the relationship between dose (arsenic) and its resulting response. The data thus obtained from heavy metal analysis was statistically analyzed. ANOVA was used to statistically analyzed ($p-value$) the differences among different parameters under studied.

Table 1. Commercially available kits for the measurement of Serum biochemical parameter

Serum biochemical parameter	Kit Company	Reference number/ Catalog number
Sodium	BIOMED	Ref. # SOD100100
Potassium	BIOMED	Ref. # POT100040
Chloride	BIOMED	Ref. # CL132100
Albumin	BIOMED	Ref. # ALB100250
Total Protein	BIOMED	Ref. # TP116250
Aspartate aminotransferase	BIOMED	Ref. # GOT111100
Alanine aminotransferase	BIOMED	Ref. # GPT113100
Urea	LiquickCor	Cat # 2-223

Table 2. Probit Analysis with 95% confidence interval in *Cirhinus mrigala.*

Time	Percent	LC_{50}	SE	95.0% Fiducial CI	
				Lower	Upper
24 hours	50	41.9853	0.5933	40.8473	43.1892
48 hours	50	39.1827	0.5657	38.0874	40.3171
72 hours	50	34.4516	0.6219	33.2439	35.6932
96 hours	50	30.1526	0.5739	29.0276	31.2872

3. Results

The impact of arsenic trioxide on the concentration of serum biochemical parameters (Na^+ ion, Cl^- ion, K^+ ion, albumin, total protein, Urea, T. Glucose, AST and ALT) in *Cirrhinus mrigala* in terms of 24-hour, 48-hour, 72-hour and 96-hour was investigated with 95% confidence interval. The LC_{50} calculated after 24 hr, 48 hr, 72 hr and 96 hr were 41.9853, 39.1827, 34.4516 and 30.1526 respectively with 95% confidence interval. The lower and upper limits of confidence intervals after 24 hr, 48 hr, 72 hr and 96 hr were (40.8473-43.1892), (38.0874-40.3171), (33.2439-35.6932) and (29.0276-31.2872) respectively as depicted in Table 2.

The results of present study showed some differences in the values of serum biochemical parameters of control and arsenic exposed fish (Table: 3).

Sodium ions level in arsenic exposed fish at different durations i.e. 24hr (154.2±1.15), 48hr (150.3±0.96), 72hr (147.0±1.00) and 96hr (143.9±1.00) were lowered in comparison to mean of control group (163.0±0.59). The level of Na^+ ion decrease gradually after 24 hr (154.25.0.01), 48 hr (150.32±0.09), 72 hr (147.00±0.00) and 96 hr (143.91±0.01) respectively (P<0.01). There was also overall reduction in level of Cl^- ions in arsenic exposed groups as compared to the mean of control groups (129.9±0.50). In arsenic exposed group the level of chloride ions significantly decreased ((P<0.01) after 24 hr (129.7±1.08), 48 hr (118.7±1.01), 72 hr (103.9±0.60) and 96 hr (95.1±0.52). The albumin (3.03±0.01) and total protein (4.13±0.01) levels in control fish groups were significantly higher (P<0.01) than all arsenic exposed groups (24 hr, 48 hr, 72 hr and 96 hr). The level of albumin

decreases in arsenic exposed groups after 24 hr (2.90±0.01), 48 hr (1.95±0.01), 72 hr (1.80±0.09) and 96 hr (1.69±0.02). Time duration dependent decrease in the level of total protein was observed such as 3.87±0.02, 3.40±0.09, 3.29±0.01 and 2.90±0.00 after 24 hr, 48 hr, 72 hr and 96 hr respectively.

On the other hand, the K^+ (4.63±0.01), urea (3.02±0.02) and glucose (101.2±0.05) total protein (4.13±0.01) levels in control fish groups were significantly higher (P<0.01) than all arsenic exposed groups (24 hr, 48 hr, 72 hr and 96 hr). The concentration of potassium ions increases with increasing exposure duration i.e. after 24 hr (6.41±0.03), 48 hr (6.69±0.02), 72 hr (7.39±0.01) and 96 hr (7.98±0.01). Under 24 hr, 48 hr, 72 hr and 96 hr arsenic exposed groups, the level of urea increases i.e. 3.75±0.12, 4.06±0.03, 4.55±0.06 and 4.98±0.01 respectively. There was gradual increment in the glucose level in arsenic exposed groups of 24 hr (115.8±0.98), 48 hr (130.3±1.16), 72 hr (139.3±1.58) and 96 hr (147.8±1.10). The level of AST (95.4±0.22) and ALT (24.1±0.08) in control groups were significantly lower (P<0.01) than the arsenic exposed groups. The level of AST increased after 24 hr, 48 hr, 72 hr and 96 hr such as 98.13±0.04, 108.29±0.01, 120.44±0.05 and 135.12±0.01 respectively. Under the exposure of arsenic, the level of ALT increased after 24 hr (26.1±0.12), 48 hr (37.1±0.50), 72 hr (45.1±0.44) and 96 hr (65.9±0.56).

Hence, the obtained data indicated that the concentrations of Na^+ ion, Cl- ion, albumin and total protein in arsenic exposed group after 24 hour, 48 hour, 72 hour and 96 hour gradually decreased. This decreasing trend showed that as the duration of metal exposure increases the concentrations of Na^+ ion, Cl^- ion, albumin and total protein decrease in the respective groups. While in groups exposed to arsenic for different durations, the-

Table 3. Serum Biochemistry of arsenic exposed and control fish, *Cirrhinus mrigala.*

Treatment	Exposure Duration	Na$^+$ (mmol/L)	K$^+$ (mmol/L)	Cl$^-$ (mmol/L)	Alb (g/dL)	T. Protein (g/dL)	Urea (mg/dL)	T. Glucose (mg/dL)	AST (U/L)	ALT (U/L)
Control	24-hr	162.3±1.16a	4.62±0.01e	129.1±0.65a	3.10±0.06a	4.13±0.02a	3.03±0.02d	100.1±0.61e	95.2±0.12d	24.1±0.18e
	48-hr	162.8±0.64a	4.63±0.03e	130.0±1.26a	3.07±0.05a	4.12±0.02a	3.01±0.08d	102.5±0.61e	95.5±0.65d	24.0±0.29e
	72-hr	163.1±1.52a	4.62±0.06e	131.1±0.95a	2.97±0.01a	4.14±0.02a	3.02±0.01d	101.7±0.53e	95.2±0.41d	24.1±0.05e
	96-hr	163.7±1.78a	4.65±0.04e	129.5±1.22a	3.01±0.03a	4.13±0.01a	3.01±0.06d	100.6±0.59e	95.7±0.60d	24.1±0.07e
Mean±SE		163.0±0.59A	4.63±0.01B	129.9±0.5A	3.03±0.01A	4.13±0.01A	3.02±0.02B	101.2±0.05A	95.4±0.22B	24.1±0.08B
Arsenic	24-hr	154.2±1.15b	6.41±0.05d	129.7±1.08a	2.90±0.01a	3.87±0.04b	3.75±0.12c	115.8±0.98d	96.1±0.66d	26.1±0.12d
	48-hr	150.3±0.96bc	6.69±0.05c	118.7±1.01b	1.95±0.01b	3.40±0.05c	4.06±0.03c	130.3±1.16c	108.3±1.00c	37.1±0.50c
	72-hr	147.0±1.00cd	7.39±0.05 b	103.9±0.60c	1.80±0.09bc	3.29±0.04c	4.55±0.06b	139.3±1.58b	120.4±1.71b	45.1±0.44b
	96-hr	143.9±1.00 d	7.95±0.03 a	95.1±0.52d	1.69±0.02c	2.90±0.03d	4.98±0.01a	147.8±1.10a	135.1±1.72a	65.9±0.56a
Mean±SE		148.8±1.24B	7.11±0.18A	111.8±4.0B	2.09±0.1B	3.36±0.10B	4.33±0.14A	133.3±3.6A	115.4±8.8A	43.6±4.3A

Note: Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall me

level of K+, urea, total glucose, AST and ALT increases after 24-hour, 48-hour, 72-hour and 96-hour.

4. Discussion

Heavy metals contaminating the water bodies are considered as major threat for the fish. Fish has the ability to accumulate metals to such an extent that it causes alteration in biochemical, antioxidant and physiological parameters of blood etc [51].

The results reported by [35] observed a significant decrease (P<0.05) in Na+ plasma level in *Prochilodus lineatus* exposed to the highest concentration of lead (71 mg/L) after 48 hours. The results of [36] reported that Plasma Na$^+$, Plasma Cl$^-$ and protein levels decreased in *Oreochromis niloticus* as exposure time of copper toxicity increases (3, 7, 14 and 21 days). At 40 μ g/ L and 400 μ g/ L of copper, maximum decrease of about 14 % and 29 % respectively were observed at 21 day of exposure. While plasma glucose concentration increased by about 104 % at 3 day and 239 % at 14 day of exposure to 40 μ g/ L and 400 μ g/ L of Copper respectively. Similarly, the data obtained from present study also depicted that the plasma levels of Na$^+$, Cl$^-$ and total protein decreases in *Cirrhinus mrigala* as the arsenic exposure time increases. On the other hand, plasma glucose level increases as metal exposure duration increases. Thus, all these results are in agreement with the present study. In the serum of *Oreochromis niloticus*, Na$^+$ and Cl$^-$ levels were decreased whereas the level of K$^+$ increases after metals (Ag, Cd, Cr, Cu, Zn) exposure [37]. It was found by [38] that Chloride levels decreased significantly (116.4±1.45) after one-week of exposure to sublethal concentration (10 mg/L) of cyfluthrin to *Cyprinus carpio* as compared to 48-hour exposure (123.2±2.27). These results are also in accordance with our present study's result which also show that Cl$^-$ level decreases significantly (p<0.01) in *Cirrhinus mrigala* as the arsenic exposure duration increases.

Decrease in total protein level in *Labeo rohita* exposed to hexavalent chromium as the duration of exposure increases but the decrease was significant only at the end of 96hour of exposure [39]. Effect of cadmium exposure on glucose

concentration in *Cyprinus carpio* was investigated by [40]. They noted that glucose level in blood of cadmium exposed fish was found to be increase significantly (P < 0.05) as compared to the glucose level determine in the control groups. It was concluded that intense stress was caused by high metal concentration than low metal concentration, leading to additional increment in glucose level at 7 and 14-day [41].

The results similar to the present work were also investigated by [42]. *Clarias gariepinus was exposed to* sublethal mercury chloride concentrations (0.04 and 0.12 ppm) for 7, 14, 21 and 28 days with supplementation of selenium and vitamin E. The mean concentration of Urea, AST, ALP, ALT Na+ and Creatinine were increased significantly as compared to the control values. The time of exposure main effects was significant with positive correlations, recorded between Na+ and K+ (r Na+=0.999 and r K+=0.997), Urea and Creatinine (r Urea=0.839 and r Creat=0.829) and between ALP, ALT and AST in the four periods (r ALP=0.997, r ALT =0.962 and r AST=0.984) respectively.

Present study reported increase in the levels of AST and ALT as toxicity exposure time increases. These results were in agreement with [43]. They also reported that the activities of serum ALT and AST in *Oreochromis niloticus* on exposure to Zinc and Cadmium increases significantly as compared to control. Similarly, AST and ALT concentrations increases significantly in Zinc exposed *Oreochromis niloticus* [44].

The study done by [48] that the Alb level in *Catla catla* exposed to heavy metals (Ni and Zn) decreased as the toxicity exposure duration increases comparing to the control group.

5. Conclusion and Recommendations

The present study results demonstrated that arsenic (197.84 g/mol) exposure caused significant (p<0.01) duration dependent alterations in serum biochemical parameters in *Cirrhinus mrigala* considered to be highly toxic. The level of serum biochemical parameters varied with exposure period such as the levels of Na$^+$, Cl$^-$, albumin and total protein decreases whereas the levels of K$^+$, urea, total glucose, AST and ALT increases with increasing exposure duration (Table:3).

This alteration is mainly, due to oxidative stress induced by trivalent arsenic. As fish are very important food source in Asian countries so it is necessary to convert the more toxic form of arsenic i.e. trivalent arsenic to less toxic form i.e. pentavalent arsenic before emission into the aquatic ecosystems. This conversion is important because the toxicity of arsenic trioxide is due to its high solubility in water as compared to arsenic pentaoxide that make it readily available to fish. One way for this conversion is pH, as it plays an important role in determining which arsenic form will occurred in groundwater. Usually, under reducing conditions more trivalent arsenic is found in groundwater. On the other hand, under oxidizing conditions the pentavalent arsenic prevails in groundwater [49]. Therefore, by controlling pH the formation and occurrence of different forms of arsenic can be control in natural water bodies. This study has investigated and discussed the effect of arsenic trioxide exposure on serum biochemical parameters of *Cirrhinus mrigala* for specific duration and concluded that fish serum biochemical parameters could be used as sensitive biomarker in aquatic toxicological studies and in assessing health of fish. But there is need to address the toxicological effects of arsenic in the presence of other toxicants in aquatic ecosystem along with investigation of whether fish species, age, sex and season influence arsenic toxicity or not.

Conflicts of Interest

The author declared that they have no conflicts of interest.

Data Availability statement

The data presented in this study are available on request from the corresponding author.

Author Contributions

All authors participated in the initial draft creation, reviewed the manuscript, and contributed other editing process.

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