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Research Article

ALTERATIONS IN BIOCHEMICAL PARAMETERS INDUCED BY AS_2O_3 AND THEIR RECOVERY IN GRASS CARP, *CTENOPHARYNGODON IDELLUS*

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ABSTRACT
Chronic toxicity assays using arsenic trioxide were performed on grass carp, *Ctenopharyngodon idellus*, to evaluate their impact on protein content, cholesterol and enzymes like ACP, ALP, GOT and GPT. The total protein showed a continuous decline from 15th day (13.16 mg/ml) to 60th day (12.32 mg/ml) of exposure whereas there was no significant change in the cholesterol content and weight of the fish. Significant decrease in the activities of ACP, ALP, GOT and GPT were recorded with increase in the exposure period. The total protein and enzymes recovered in 60 days of recovery period.

Keywords: *Arsenic Trioxide, Fish, Enzymes, Protein*

INTRODUCTION
Arsenic contamination of ground water has become an acute problem in recent years, causing serious poisoning to large number of people throughout the world. The level of arsenic is rising in some agrarian states in India like Punjab, where it ranges from 3.0 to 85 ppb in ground water (Hundal *et al.*, 2009) which is well above the recommended limits by World Health Organization (2001), which put the safe limits at 10ppb for drinking water. The trivalent form of arsenic, arsenic trioxide is considered more toxic because of its tendency to accumulate more in freshwater aquatic organisms. Most of the toxicological investigations on fish have been done on physiological (Radhaiah *et al.*, 1990), biochemical (Vutukuru *et al.*, 2007 and Kandemir *et al.*, 2010) and Ultrastructural aspects (Abdel-Moneim and Abdel-Mohsen 2010). In many of the investigations, enzymes have been used as biomarkers of heavy metal pollution (Ruparelia *et al.*, 1992; Humtsoe *et al.*, 2007 and Kumari *et al.*, 2011) only for the exposure period of the toxicant and not looking at their recovery after exposure to the toxicant. The present investigation have been done to observe the effects of sub-lethal concentration of arsenic trioxide on the total protein, cholesterol, acid and alkaline phosphatase (ACP and ALP) as well as on glutamate-oxaloacetate and glutamate-pyruvate transaminase (GOT and GTP) in the liver of *Ctenopharyngodon idellus* both for exposure and recovery periods.

MATERIALS AND METHODS
The test fish, *Ctenopharyngodon idellus*, both sexes, were brought to the laboratory from Nanoki fish farm, Fathegarh Sahib, Punjab (India) and were acclimatized to the laboratory conditions for 14 days under normal photoperiod and constant temperature (22°C to 24°C) conditions. They were fed with artificial diet (Tokyu floating type fish food) having nutrients crude protein (32%), crude fat (4%) crude fibre (5%), crude ash (10%) moisture (91%) and nitrogen free extract (31%). Arsenic Trioxide (As_2O_3), manufactured by Qualikems Fine Chemical Pvt. Ltd. New Delhi, was used for the present toxicological investigation. The stock solution was prepared by dissolving 1.32g of As_2O_3 and 4g NaOH in one litre of distilled water. 1ml of stock solution contained 1mg of As. LC_{50} for 96hr value of As for *Ctenopharyngodon idellus* was found to be 17.24 mg/L by probit analysis (Finney 1980). The experimental fish were exposed to 3.45mg/L of As which is 1/5th concentration of LC_{50} value, along with control group. The experiment was carried out in triplicate with 12 fish each with average length of 14.83 (± 3.67) cm and weight 19.31 (± 2.52) g. After exposure, the fishes were dissected to sample out liver on 15th, 30th, 45th and 60th day for further analysis. Weight of fishes was also recorded. A recovery period of

60 days was also given immediately after the exposure period (60 days). During the recovery period, the fish was fed on the same diet and no exposure of the toxicant was given. The fish were dissected on 90th and 120th day.

Tissues were weighted and 10% homogenate were prepared in ice cold 0.9% saline solution. The total protein and total cholesterol were estimated by Lowry's method (Lowry *et al.*, 1951) and Zlatkis method (1953) respectively. The enzymes, Acid and Alkaline Phosphatases (Bergmeyer 1963); Glutamate-Oxaloacetate and Glutamate-pyruvate Transaminase (Reitman and Frankel 1957) were also analysed. Data was analysed for significance by student't' test by using Graphpad software (Quick calculus online calculator).

RESULTS AND DISCUSSION

The concentrations of total protein, cholesterol, ACP, ALP, GOT and GPT in grass carp on exposure to sub-lethal concentration of arsenic trioxide is shown in Table 1 both for exposure and recovery periods. There is no significant change in the weight of the fish during exposure and recovery period (Figure 1a), whereas according to Khan *et al.*, (2004), abnormalities in biochemical parameters because of mercury intoxication result in decreased growth rate in *Ctenopharyngodon idellus*.

Table 1: Biochemical parameters of *Ctenopharyngodon idellus* (Cuvier and Valenciennes, 1844) for exposure (3.45mg/L of Arsenic) and recovery periods

Parameters	Control	Treatment Days				Recovery	
	0	**15**	**30**	**45**	**60**	**90**	**120**
Weight (g)	19.31 ±2.52	17.53 ±3.39	16.77 ±1.69	17.33 ±3.32	17.22 ±3.47	15.30 ±4.61	17.65 ±3.12
Protein (mg/ml)	13.25 ±0.75	13.16 ±0.8	*11.91 ±0.54	*11.07 ±0.27	*12.32 ±0.31	*12.73 ±0.58	13.61 ±0.79
Cholesterol (mg/ml)	3.12 ±0.02	3.06 ±1.07	3.43 ±1.18	3.48 ±0.93	3.51 ±1.02	3.43 ±1.27	3.16 ±0.80
ACP (U/mg protein)	0.31 ±0.02	0.31 ±0.05	*0.13 ±0.07	*0.09 ±0.05	*0.08 ±0.07	*0.27 ±0.008	*0.27 ±0.01
ALP (U/mg protein)	0.72 ±0.005	0.69 ±0.08	0.66 ±0.06	*0.62 ±0.08	*0.55 ±0.07	*0.67 ±0.06	*0.69 ±0.01
GOT (U/mg protein)	19.19 ±0.47	17.75 ±2.87	*14.78 ±2.33	*11.69 ±1.33	*8.53 ±2.16	*16.03 ±1.78	*17.33 ±0.99
GPT (U/mg protein)	9.61 ±0.65	8.65 ±1.24	*6.70 ±0.68	*1.96 ±2.19	*0.59 ±0.55	*5.26 ±0.98	*7.58 ±0.68

ACP=Acid phosphatase; ALP=Alkaline phosphatase; GOT=Glutamate-oxaloacetate transaminase; GPT= Glutamate-pyruvate transaminase
** Significant w.r.t. control (P< 0.05)*

The total proteins showed a significant decline after 15th day (13.16 mg/ml) of exposure (Figure 1b) and the changes were significant on 30th (11.91mg/ml), and on 45th day (11.07 mg/ml) with respect to control (P<0.05). The protein content showed significant increase from 45th to 60th day (12.32 mg/ml) of exposure period. During the recovery period i.e. 60th day onwards, the proteins showed recovery and reached almost equal to the control values (13.25 mg/ml) on 120th day (13.61 mg/ml). During stress condition, fish uses protein as source of energy to meet the increased energy demand to detoxify the toxicant (Vutukuru 2005). The other reasons for depletion of tissue protein can be due to impaired rate of protein synthesis (Rajamanickam and Muthuswamy 2008) or decrease in uptake of amino acid into polypeptide chain (Kori-Siakpere and Ubogu 2008). There was no significant change in the cholesterol level (Figure 1c). There are conflicting reports about the changes in cholesterol content upon exposure to heavy metals with

some reporting decrease (Virk and Sharma 1999) and others reporting increase (Meenakumari *et al.*, 2010).

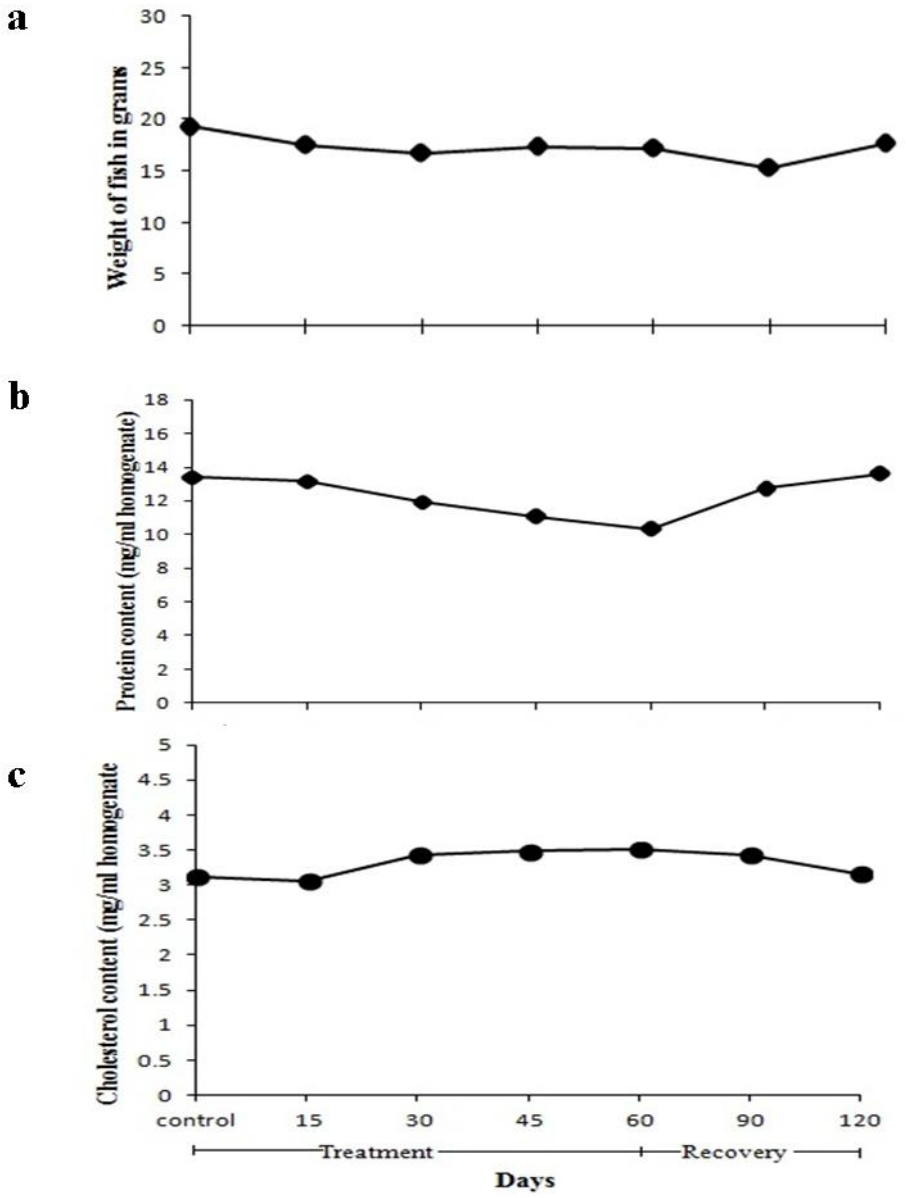

Figure 1: Effects of arsenic trioxide on a) total weight, b) Protein content and c) cholesterol content. Data is presented as mean value of 6 fish

A decrease in acid phosphatase (ACP) and alkaline phosphatase (ALP) was observed (Figure 2a) during exposure period, increasing with the duration. The decrease is significant ($P< 0.05$) with respect to control (0.31 U/mg protein) on 30^{th} (0.13 U/mg protein), 45^{th} (0.09 U/mg protein), 60^{th} day (0.08 mg/mg protein) while a significant increase was observed during recovery period with respect to exposure period. In alkaline phosphatase decrease was significant ($P<0.05$) on 45^{th} (0.62 U/mg protein) and 60^{th} day (0.55 U/mg protein) and during recovery period also a significant increase was found on 120^{th} day (0.69 U/mg protein) as compared to exposed fish and came close to control fish (0.72 U/mg protein). This may be due to destruction of hepatocytes or decrease in the enzyme production or increased cell membrane

permeability. Humtsoe *et al.,* (2007) observed that accumulation of arsenic in liver in *Labeo rohita* could be possible reason for variation of enzyme activities. Sreekala and Zutshi (2010) noticed decrease in acid and alkaline phosphatase activity in fish inhabiting natural water which receiving effluents from pharmaceutical industry. Mariappam and Karuppasamy (2014) also observed marked inhibition of ACP and ALP activity in all tissues of Cu and Cd treated fish may be due to leakage of enzymes from the damage tissue.

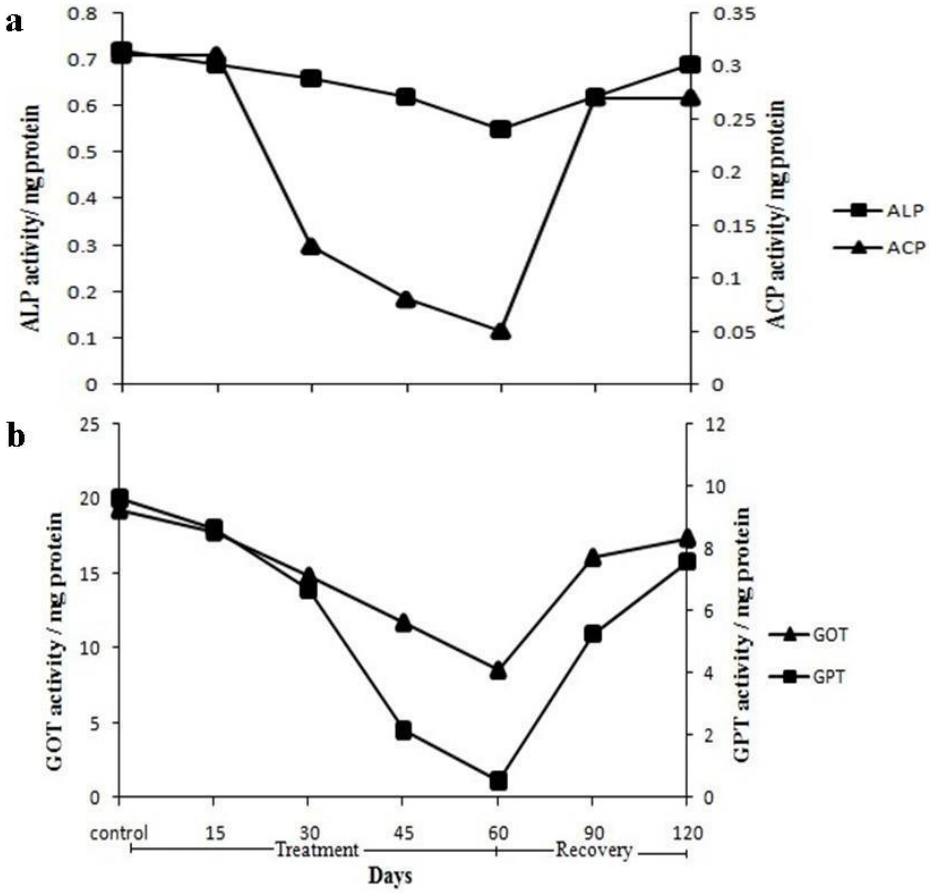

Figure 2: Effects of arsenic trioxide on a) the ACP, ALP activity and b) GOT, GTP activity. Data is presented as mean value of 6 fish

The present data demonstrated a significant decrease in the GOT and GPT activity in liver during treatment (Figure 2b). In GOT a significant change ($P<0.05$) was noted on 30th (14.78 U/mg protein), 45th (14.78 U/mg protein), 60th (8.53 mg/mg protein) day which showed decrease w.r.t. control (19.19 U/mg protein). On 90th day (16.03 U/mg protein) and 120th day (17.33 U/mg protein) reverse change in the values of enzyme activity was noticed. Similarly, in case of GTP significant decrease was noticed on 30th (6.70 U/mg protein), 45th (1.96 U/mg protein) and 60th day (0.59 U/mg protein) while increase was observed on 90th day (5.26 U/mg protein) and 120th day (7.58 U/mg protein) w.r.t. control (9.61 mg/mg protein). These changes might be due to altered protein content in the liver and damage in the liver tissue (Humtsoe *et al.,* 2007), however, Bakthsalam and Ready (1984) reported increase in the enzyme activities on exposure to pesticides indicating incorporation of amino acids into the TCA cycle to overcome the stress of exposure. Al-Ghanim (2012) noted gradual decrease in protein whereas elevation in cholesterol level and activity of ALT in *Oreochromis niloticus* exposed to Malathion. Vasanth *et al.,* (2012) reported increase in the activities of AST, ALT and ALP in the liver of *Labeo rohita* (Hamilton, 1822) upon

exposure to anthracene. Das *et al.*, (2012) studied Toxicological effects of arsenic on *Channa punctatus* and observed that total protein showed significant decrease w.r.t control, activities of ALT and AST also found to be decreased and concluded that the toxicants can bring about distortions in the cell organelles, which may bring about elevation or inhibition in the activities of the enzymes. It can be concluded from present study that enzyme activities, total protein and total cholesterol are significantly altered after exposure to arsenic and the effects are dependent on duration of exposure and if fish are kept in the arsenic free water, enzymatic changes can be reversed.

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REFERENCES
Abdel-Moneim AM and Abdel-Mohsen HA (2010). Ultrastructural changes in hepatocytes of catfish *Clarias gariepinus* from lake Mariut, Egypt. *Journal of Environmental Biology* **31** 715-720.
Bakthsalam R and Ready YS (1984). Lipid kinetics in relation to the toxicity of three pesticides in climbing perch, *Anabas testudineus* (Bloch). *Proceedings of the Indian National Science Academy B* **47**(5) 670-675.
Bergmeyer HU (1963). *Methods of Enzymatic Analysis* (Acadmic press, New York).
Finney DJ (1980). *Probit Analysis* (Cambridge University Press, London).
Humtsoe N, Davoodi R, Kulkarni BG and Chavan B (2007). Effect of arsenic on the rohu carp, *Labeo rohita* (Hamilton, 1822). *Raffles Bulletin of Zoology* **14** 17-19.
Hundal HS, Singh D and Singh D (2009). Arsenic content in ground and canal water of Punjab, North-West India. *Environmental Monitoring and Assessment* **154** 393-400.
Kandemir S, Örün I, Talas Z, Örün GN, Erdoğan K, Işık M, Altaş L and Duran A (2010). Effects on mortality of biochemical and limnological properties on some fish species in Sultansuyu dam lake (Malatya), Turkey. *Turkish Journal of Fisheries and Aquatic Sciences* **10** 431-437.
Khan MS, Khan SA, Chaudhary ZI, Khan MN, Aslam A and Ashsaf K (2004). Mercury intoxication in grass carp (*Ctenopharyngodon idellus*). *Pakistan Veterinary Journal* **24**(1) 33-38.
Kori-Siakpere O and Ubogu E (2008). Sublethal haematological effects of zinc on the fresh water fish, *Heteroclarias spp*. (Osteichthyes: Clariidar). *African Journal of Biotechnology* **7** 2068-2073.
Kumari K, Rajan N and Sinha RC (2011). Multiple biomarker response in the fish, *Labeo rohita* due to hexavalent chromium. *2nd International Conference on Biotechnology and Food Science* **7** 155-157.
Lowry OH, Rosenberg NG, Farr AL and Randall RJ (1951). Protein measurement with folin-phenol reagent. *Journal of Biological Chemistry* **193** 265-275.
Mariappan M and Karuppasamy R (2014). Effect of individual and co-exposure of copper and cadmium on phosphatise activity in freshwater fish, *Cyprinus carpio*. *International Journal of Modern Research and Reviews* **2**(3) 116-121.
Meenakumari S, Showkat AP and Saravanan TS (2010). Ambient copper induced alterations in hematology and biochemistry of the major carp, *Labeo rohita* (Hamilton). *International Journal of Integrative Biology* **10**(2) 119-123.
Radhaiah V and Jayantha KR (1990). Toxicity of the pyrethroid insecticide fenvalerate to a fresh water fish, *Tilapia mossambica* (Peters): Changes in glycogen metabolism of muscle. *Ecotoxicology* and *Environmental* Safety **19**(1) 116-121.
Rajamanickam V and Muthuswamy N (2008). Effect of heavy metals induced toxicity on metabolic biomarkers in common carp (*Cyprinus carpio* L.). *Maejo International Journal of Science and Technology* **2** 192-200.
Reitman S and Frankel SA (1957). Calorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology* **28** 56-60.

Ruparelia SG, Verma Y, Mehta NS and Rawal UM (1992). Cadmium accumulation and biochemical alterations in the liver of freshwater fish *Sarotherodon mossambica* (Peters). *Bulletin of Environmental Contamination and Toxicology* **2**(2b) 129-136.

Sreekala G and Zutshi B (2010). Acid and Alkaline phosphatase activity in the tissues of *Labeo rohita* from freshwater lake of Bangalore. *The BioScan* **2** 365-372.

Virk S and Sharma RC (1999). Biochemical changes induced by nickel and chromium in the liver of *Cyprinus carpio* L. *Pollution Research* **18**(3) 217-222.

Vutukuru S (2005). Acute effects of hexavalent chromium on survival, oxygen consumption, hematological parameters and some biochemical profiles of the Indian major carp, *Labeo rohita*. *International Journal of Environmental Research and Public Health* **2** 456-462.

Vutukuru S, Prabhath NA, Raghavender M and Yerramilli A (2007). Effect of arsenic and chromium on the serum amino-transferases activity in major carp, *Labeo rohita*. *International Journal of Environmental Research and Public Health* **4**(3) 224-227.

WHO (2001). Arsenic in Drinking water No.210, Geneva. Available: http://www.who.inf/inf-fs/en/fact210.html.

Zlatkis A, Zak B and Boyle AJ (1953). A new method for the direct determination of serum cholesterol. *Journal of Laboratory and Clinical Medicine* **41**(3) 486–490.