RESEARCH ARTICLE

Impact of Heavy metal, Arsenic trioxide on Biochemical profile of teleost, *Clarias batrachus* (Linn.)

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ABSTRACT

The present investigation aims at evaluating the toxic effect of heavy metal, Arsenic trioxide on biochemical profile of *Clarias batrachus* after 30, 45 and 60 days of post treatment with experimental chemical , arsenic trioxide. Lc 50 value calculated for sublethal study of arsenic trioxide was 8.7 mg/l. Following biochemical parameters were analysed: Serum Protein, Serum Cholesterol. Serum Glucose, Acid phosphatase and Alkaline phosphatase**.** Decrease in serum protein observed in present study was due to liver cirrhosis or nephrosis. Increase in cholesterol indicated environmental stress in *Clarias batrachus* .The significant reduction in plasma glucose levels during acute treatment indicates hypoxic condition. Increased stimulation of alkaline phosphatase corresponds to pathological processes as liver impairment, kidney dysfunction and bone disease.

Keywords: Arsenic trioxide, *Clarias batrachus*, Lethal concentration , Acid phosphatase, Alkaline phosphatase.

INTRODUCTION

The effect of heavy metals on aquatic organism is currently attracting wide spread attention particularly in studies related to industrial pollution. High toxicity of industrial pollutions have been known since long time, but their hazardous nature as pollution of aquatic environment has been matter of concern only after a large number of deaths of fishes occurring in different areas due to different metals. In aquatic environment, fishes are usually regarded as organisms of choice for assessing the effects of environmental pollution on aquatic ecosystems

Gernhofer *et al.* (2001). Despite progress made in environmental waste management, heavy metals still pose immense health hazards to humans and biota unlike other classes of pollutants, which can be biodegraded and destroyed completely. The name "Arsenic" is derived from the Greek word "arsenikon", which means yellow orpiment. Arsenic compound have been mined and used since ancient times. The extraction of the element from arsenic compound was first reported by Albertus Magnus in 1250 A.D. Emsley (2001**)** Arsenic, a heavy metal ranks 20th in earth's crust, 14th in sea water and 12th in human body. Arsenic exhibit metallic as well as non-metallic properties. Arsenic is aknown chemical element that has the symbol 'As' and atomic number 33. Its atomic mass is 74.92 and is prevalent in the environment, occurring both naturally and as a result of environment pollution.

Sources of arsenic include treatment of wood using chromate copper arsenate, burning of coal in thermal power plants, operation of gold minning, as treatment of land with arsenical pesticides. Arsenic occurs naturally and its use is possibly aggravated by the use of over powering aquifers and by phosphorous from fertililizers, production of dyes from tanneries, application of some herbicides and insecticides. It is present in effluents from Laundring Tamaki and Frankenbeger (1992) Arsenic, an important environmental contaminant, is present in the aquatic environment as a result of geogenic and anthropogenic processes, Gonzalez *et al.* (2006); Singh and Banerjee (2008). Biochemical characteristics of blood are among the important indices of the status of internal environment of the fish organism (Luskova, 1997). The present study focuses on the impact of arsenic on biochemical profile of *Clarias batrachus* .Arsenic generally exists in the inorganic form in water samples. Under different redox conditions arsenic is stable in the +5, +3, -3, and 0 oxidation states. The pentavalent (+5) arsenic or arsenate species include AsO43-, and H2AsO4-. The trivalent (+3) arsenic or arsenite species include As (OH)4-, AsO2(OH)2-, and AsO-3. The pentavalent arsenic species are predominant and stable in the oxygen-rich aerobic environment, whereas the trivalent arsenic species are predominant in the moderately reducing anaerobic environment such as groundwater.

MATERIALS AND METHODS

Test fish:

Healthy living specimen of teleost, *Clarias batrachus* were collected from local fish market of Meerut. Fish measuring 15 ± 2cm in length and 60 ± 8 gm in weight were selected for the present study. Selected fishes were acclimatised to the laboratory conditions for period of 15 days.

Preparation of stock solution and determination of 96 hr LC 50 value of Arsenic trioxide:

1gm of arsenic trioxide stock solution was prepared by dissolving arsenic trioxide in 1N HCl under constant heating. The pH was adjusted to 7.4 by adding 1N NaOH dropwise and the solution was filtered by passing through filter paper. For the determination of median tolerance limits or LC 50, different concentrations of arsenic trioxide (20, 30, 40, 50, 60, 70, 80 and 90 mg/l) were prepared from the stock and added in separate glass aquaria containing 50 L of water.

Chemical exposure and Experimental design:

Fishes were divided into 4 equal groups each comprising of 30 fishes. Each group was kept in separate glass aquaria of 250 litre capacity. First group was treated as control group. Fishes of other 3 groups were treated with sub-lethal concentration 8.7mg/l arsenic trioxide for period of 30, 45 and 60 days. Water in the aquariums were renewed after 24 hours and fresh solution of the toxicants were added to bring the concentration to the desired level.

Biochemical studies:

All biochemical studies were performed with the serum of control as well as treated groups of fishes.

Preparation and preservation of serum:
Fish blood was centrifuged at the speed of 3000 rpm. The serum was separated and preserved in the refrigerator at -20^{0}C in the deep freezer. These vials were properly labelled according to the experimental design. Whenever the serum was required, it was first of all brought to the room temperature and then further estimations were done.

1. Determination of Serum Protein
Total serum protein was determined by Kjeldahl's digestion

2. Determination of Serum Cholesterol
Serum cholesterol was estimated with the help of one step method (Wybenga and Pilleggi).

3.Determination of Glucose
Glucose level was estimated by Kit method (End point o-toludine).

4. Determination of Acid Phosphatase – According to Kind and King's kit method.

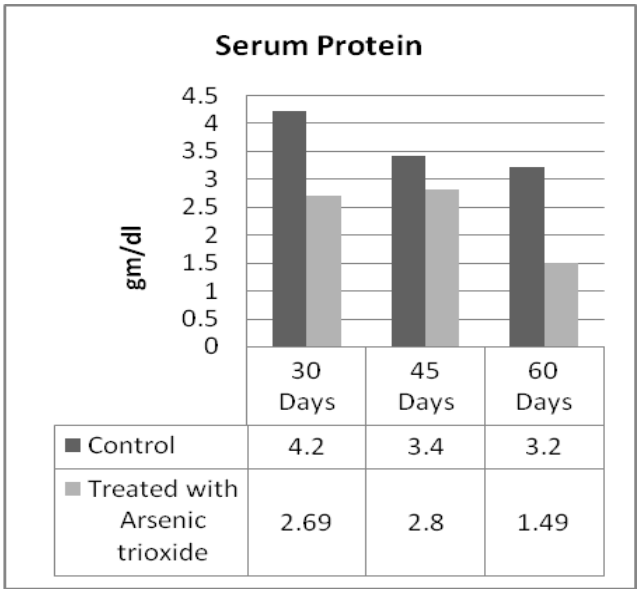
5. Determination of Alkaline Phosphatase:
For the estimation of serum alkaline phosphatase Kind and king's

RESULTS AND DISCUSSIOINS

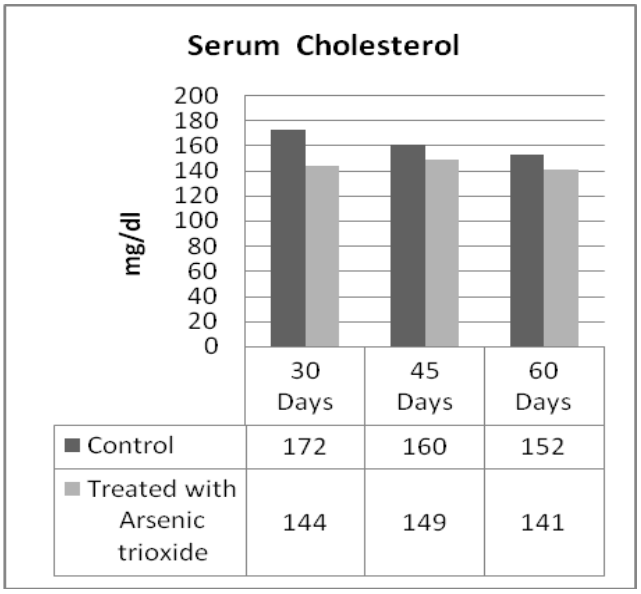
The Serum protein was observed to be 2.69 gm/dl after 30 days of PT with arsenic trioxide. The observed value showed difference in parameter when ($p<0.05$), compared with control values after 30 days . The Serum protein showed increase of 2.80 gm/dl after 45 days of PT with arsenic trioxide. While this parameter showed decline of 1.49 gm/dl after 60 days of PT with arsenic trioxide.

In the present investigation the decrease in Serum protein during acute and sublethal treatment is supported by the reports of Nandi *et al.*, 2005. Palaniappan and Vijayasundaram (2009) suggesting that the decrease in plasma protein may be due to liver cirrhosis or nephrosis or might be due to alteration in enzymatic activity involved in protein biosynthesis. Pazhanisamy (2002) reported change in total protein content of various tissues in different fishes exposed to different heavy metals. Jana and Bandyopathyay (1981); Jatyajit (1996); Baskaran and Palanichamy (1995) have reported such a reduction in protein content when the fish *Channa punctatus* has been exposed to heavy metals such as mercury, arsenic and lead and *Channa striatus* exposed to mercury cadmium and lead. Gagnon *et al.* (2006) reported that due to metal complex formation, normal functioning of cell is disturbed and that in turn may result in variation on physiological and biochemical mechanisms of animals. Serum Cholesterol was observed to be 144mg/dl after 30 days of PT with arsenic trioxide. The observed value was found to decline when ($p<0.05$), compared with control values after 30 days. This parameter showed increase 149 mg/dl after 45 days of PT with arsenic trioxide. 60 days of PT with arsenic trioxide showed decline of 141 mg/dl. The observed value showed decrease when ($p<0.05$), compared with control values after 60 days. Serum Cholesterol showed initial increase after 45 days and decline was noted after 60 days. Heavy metals are known to have hazardous effects on cell structure, especially on the membranes. Therefore, it becomes evident that increase in cholesterol may be the indications of environmental stress. The present findings are in agreement with studies of Murray (1991); Gill and Epple (1993); Sastry and Shukla (1994) who pointed that hyper cholestrolemia observed in *Clarias* may be due to impairment of liver and inhibition of enzymes, which converts cholesterol into bile acid.

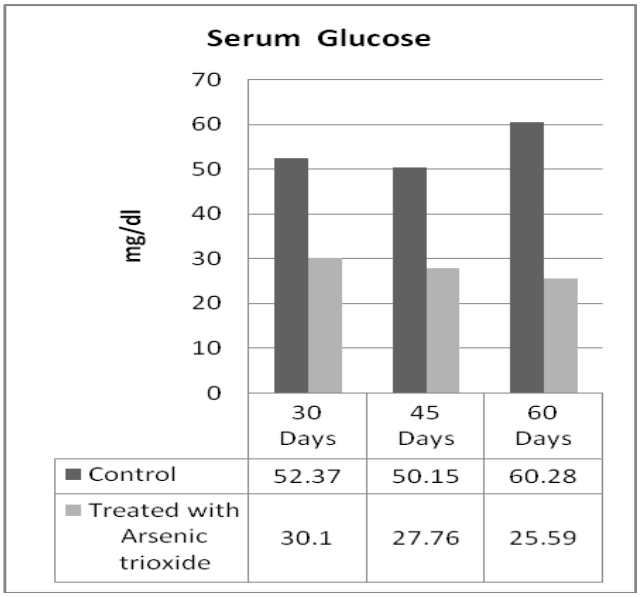
Serum Glucose was observed to be 30.10 mg/dl after 30 days of PT with arsenic trioxide. The observed value was found to decline when ($p<0.05$), compared with control values after 30 days. This parameter showed decline of 27.76 mg/dl after 45 days of PT with arsenic trioxide. While after 60 days of PT with arsenic trioxide



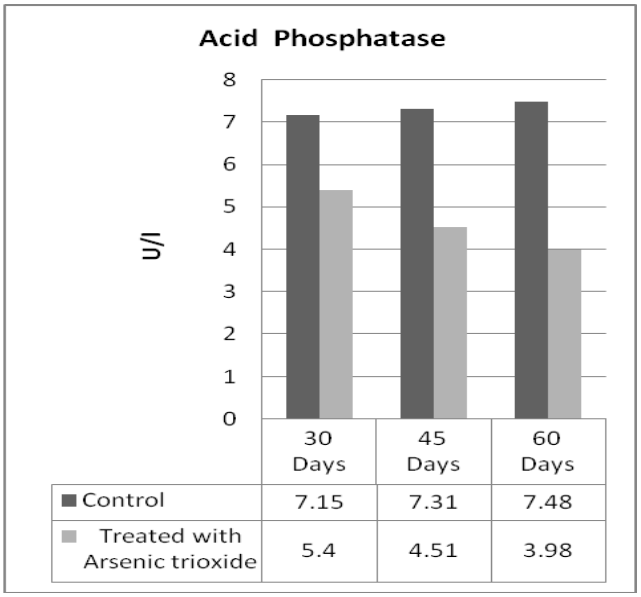
	30 Days	45 Days	60 Days
Control	4.2	3.4	3.2
Treated with Arsenic trioxide	2.69	2.8	1.49



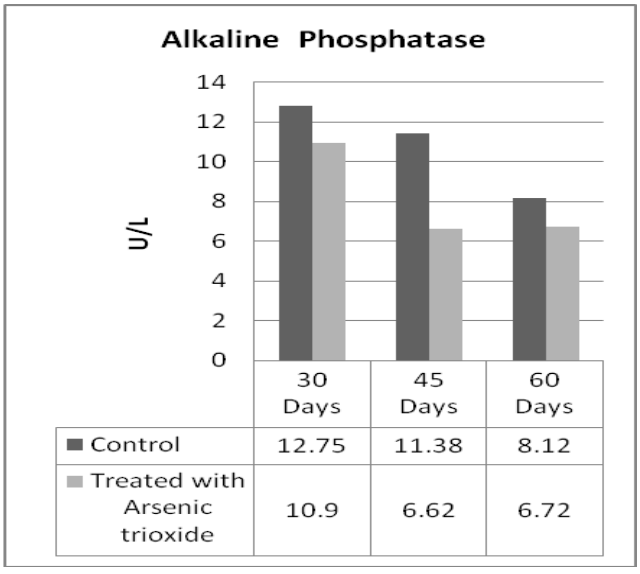
	30 Days	45 Days	60 Days
Control	172	160	152
Treated with Arsenic trioxide	144	149	141



	30 Days	45 Days	60 Days
Control	52.37	50.15	60.28
Treated with Arsenic trioxide	30.1	27.76	25.59



	30 Days	45 Days	60 Days
Control	7.15	7.31	7.48
Treated with Arsenic trioxide	5.4	4.51	3.98



	30 Days	45 Days	60 Days
Control	12.75	11.38	8.12
Treated with Arsenic trioxide	10.9	6.62	6.72

this parameter showed further decline of 25.59mg/dl. The serum glucose showed decline after 45 and 60 days of exposure period Very little attention is paid on effect of arsenic on Serum glucose level in *Clarias batrachus* . Tseng., 2004 reported that chronic exposure of arsenic or its methylated metabolites induced diabetes mellitus in rats and this condition may be responsible for hyperglycemia. Thus an elevation of blood glucose level in the present study during sublethal treatment might be due to gluconeogenesis to provide energy for the increased metabolic demands imposed by arsenic stress. The significant reduction in plasma glucose levels during acute treatment might be

due to hypoxic conditions caused by arsenic leading to an excess utilization of stored carbohydrates.

Acid Phosphatase was observed to be 5.40U/L after 30 days of PT with Arsenic trioxide. The observed value was found to decline when ($p<0.05$), when compared with control values after 30 days of exposure period. After 45 days of exposure period the observed value showed decline of 4.51 U/L, further decline in parameter was observed to be 3.98U/L after 60 days of PT with arsenic trioxide. Sastry and Gupta (1979) reported elevation in activity of acid phosphatase in *Channa punctatus* under lead exposure. The rise in the activities of acid phosphatase due to lead toxicity leads to hepatocellular damage in the organism Sharma (1999). This increase is associated with liver damage as this enzyme is known to be associated with lyososomal activity. It has been suggested that the acid phosphatase elevation causes proliferation of lysosomes in an attempt to sequester the toxic xenobiotic (Gill and Epple, 1992).

Alkaline phosphatase was noted to be 10.90U/L after 30 days of PT with arsenic trioxide. This value was found to be declined when ($p<0.05$), compared with control values after 30 days. This parameter showed decline of 6.62 U/L after 45 days of PT with arsenic trioxide. The observed value showed decline in parameter when ($p<0.05$), compared with control values after 45 days. Alkaline phosphatase showed increase of 6.72U/L after 60 days of PT with arsenic trioxide. Since very less work has been reported directly on this metal but the findings of present work coincides with findings of workers on other heavy metals. The result is in agreement with findings of Agarwal and Sastry (1979) who have observed significant increase in activity of ALP in *Channa punctatus* after 96 hr of post treatment with mercuric chloride. Gill *et al.*, 1991 and Ranjeeta (2008) recorded an increase in alkaline phosphatase activity in *Puntius conchonius* and *Clarias batrachus* under mercuric chloride intoxication and endosulfan exposure. Ilyas et al., (2007) also noticed the same result in *Labeo rohita*. Such result might be due to increase in osteoblastic activity or intra and extra hepatic obstructions of biliary passage Jyothi and Narayan (1999).

CONCLUSION

In the present study variations in all biochemical parameters were recorded with duration of exposure to experimental chemicalArsenic trioxide. Thus it is indicated that heavy metal arsenic trioxide is causing harmful alterations in biochemical profile of economically important food fish, *Clarias batrachus.*

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