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Effect of sodium arsenite on liver function related enzymes of cat fish *Heteropneustes fossilis* and its chelation by zeolite

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Abstract

Arsenic has been associated with a multitude of health problems and various works to study its impact on different organisms are being done in different parts of the world. Remediation of metal toxicity in environment and animal body including man is a controversial topic. Fishes accumulate the toxicant easily through food chain and water and transfer it to humans. In the present study the impact of arsenic on enzymes of the fish *Heteropneustes fossilis* and the ability of synthetic zeolite as chelating agent were carried out. The alteration in activities of GPT, GOT and Alkaline phosphatase in the liver of *Heteropneustes fossilis* and the chelating effect of synthetic zeolite on it was studied after exposure to different concentrations of sodium arsenite for different durations. Fishes are exposed to two different concentrations of sodium arsenite (200 mL and 400 mL of 1% solution), for 3 different durations (3days, 7days and 15 days).The activities of GPT (F=46.63>14.24) and ALP (F=595.33>190.97) was found significantly elevated along with increasing concentration of sodium arsenite. The reversal of the effect on treatment with 1% solution of zeolite, along with sodium arsenite (GPT, F=47.78 > 14.24; and ALP, F=562.33 > 190.97, at 5% P) was reported. Where as the activity for GOT showed significant decrease (F= 132.12 > 49.69, at 5% P) on exposure to sodium arsenite and in this case also reversal of the effect on chelation with zeolite (F=107.21>49.69, at 5% P), was noticed.

Keywords: Adsorption, chelation, *Heteropneustes fossilis*, liver enzymes, sodium arsenite, synthetic zeolite.

INTRODUCTION

Today arsenic is considered as a serious toxicant metallic pollutant of wide health concern and is indiscriminately available in ground water by natural way and in agricultural run off and mining process by anthropogenic way (Shalat *et al*., 1996). Arsenic has its source from ground water enriched with arsenic, arsenic containing pesticides, mining operations and agriculture run off (Chakraborty *et al*., 1998). Humans are largely being exposed to arsenic through underground water, agricultural output and industrial effluents.

Arsenic contamination and consequent ill health of people have been reported by many researchers. It is postulated that skin cancer, conjunctivitis, melanosis, hyperkeratosis, renal dysfunctions, hepatic and respiratory disorders and hematological alteration are common health problems caused by arsenic intoxication (Smith *et al*., 2000; Tseng, 1977). It is classified as a group A and category 1 human carcinogen by the USEPA (1997), and the international association of research on cancer, (IARC, 1987) respectively. It is suggested that the uptake of significant amounts of inorganic arsenic can intensify the chances of cancer development, especially skin cancer, lung cancer, liver cancer and lymphatic cancer (Bhattacharya *et al*., 2004). Chronic, long-term exposure to arsenic has been demonstrated to be carcinogenic in humans by Huang *et al*., (2004).

It has been reported that heavy metals affect various biochemical parameters of fish liver (Jana & Bandyopadhyaya,

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1987). Arsenic induced biochemical changes in the liver tissues of freshwater fingerlings of fish *Labeo rohita*, was reported by Palaniappan & Vijayasamudram (2009) using Fourier transform infrared (FTIR) spectroscopy. Enzymes are biochemical macromolecules that control metabolic process of organisms, thus a slight variation in enzyme activities would affect the organism by disturbing its metabolism (Roy, 2002; Humtsoe *et al*., 2007). The effect of chronic exposure of sodium arsenite on various biochemical parameters on different organs of the mammal *Oryctolagus cuniculus* was reported by Tripathi & Kumar (2010).

The effect of arsenic on the activity levels of acid phosphatase, alkaline phospatase, glutamate-pyruvate transaminase and glutamate-oxaloacetate transaminase in muscles and liver tissues of the Carp*, Labeo rohita* were assessed by Humtsoe *et al.,* (2007). It was reported that enzymatic activities were reduced and significant variation in the activities of these enzymes was noted after exposure to arsenic.

The zeolite ($M_2/nO.Al_2O_3.xSiO_2.yH_2O$) comprises different elements and is an aluminosilicate mineral having special ability to carry water in their crystalline structure. They have a micro porous structure and are considered as effective adsorbent because of the ability to adsorb heavy metals from the contaminated water (Erdem *et al*., 2004). Zeolites are well known for their ion exchange capacity. The role of zeolites in the conversion of solid and liquid hazardous wastes into environmentally acceptable products has been demonstrated by Shevade *et al.,* (2004). Several zeolites, namely clinoptilolite, chabazite, SZP1, 13X and 5A have been identified as potential candidates for arsenic removal from water. Synthetic zeolites are useful because of their controlled and known physico-chemical properties relative to that for natural zeolites.

Faujasites are a class of synthetic zeolites that crystallizes in the cubic space group Fd 3m, with a lattice constant ranging from about 24.2-25.1Å, depending on the framework, aluminium concentration, cations, and state of hydration. There are 192 tetrahedral sites per unit cell. This zeolite is formed from 24-tetrahedra cuboctahedral units (sodalite cages), joined through hexagonal prisms (also known as double 6-rings), The structure can be viewed as the diamond structure, with the sodalite cages playing the role of carbon atoms, and the double 6-rings the role of C-C bonds. The pore structure is characterized by super cages approximately 12Å in diameter, which are linked through windows about 8Å in diameter, composed of rings of 12 linked tetrahedrals. The structure is highly useful in catalytic applications because of specific cages and pores which can access quite large molecules. (Herreros, 1995; Meier *et al.*, 1996; Kaduk & Faber, 1995).

Zeolite X (higher Al) and Y (lower Al) are most common Faujasites of which Y is highly catalytic in nature and are synthesized in the Na form. The acid catalysis which is very common requires replacing the Na cation by protons and converting the sieve into the H-form (Kaduk & Faber, 1995).

It has been observed that the H^+ and $NH4^+$ forms of the synthetic zeolites were capable of removing arsenite to 50 ppb within 15 min, which is the current permitted maximum contaminant level (MCL) for arsenic in the United States (Shevade & Ford, 2004). The fact that zeolite exchangeable ions are relatively innocuous (sodium, calcium, and potassium ions) makes them particularly suitable for removing undesirable heavy metal ions from industrial effluent waters. (Erdem *et al.*, 2004; Jain, 1999).

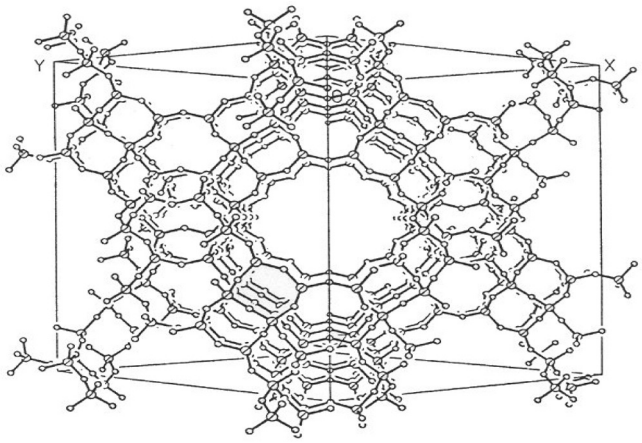


Figure 1 - The faujasite structure, The larger hatched circles represent the tetrahedral"T" sites (Al or Si), and the smaller open circles indicate the oxygen positions (Adopted from:Kaduk & Faber,1995).

In the present work the disturbance of metabolism in *Heteropneustes fossilis* on exposure to different concentrations of sodium arsenite for different durations and the ability of the synthetic zeolite, type Y, for removing arsenic toxicity was found.

MATERIALS AND METHODS

The Teleost cat fish, *Heteropneustes fossilis*, was selected for experiment and were collected from local pond. The fishes selected for experiment were 7-8 inch of length and average weight of 125-150 gm. and were maintained in glass aquarium of 76.2 cm x45.72 cm x 45.72 cm dimension, with 20 L of chlorine-free bore well water. The fishes selected for experiment were first acclimatized in the lab for 15days under normal room temperature (27-29°C), pH-7.2-7.5 and DO- 6 to 6.5 mgL^{-1}. The fishes were fed once daily with the fish food available in the market. The water was changed periodically.

The fishes were then divided into five experimental groups, each with 5 fishes. One group was kept for control set, and the remaining four for experimental sets. The test chemical selected for the experiment was obtained from SD Fine – Chem. Ltd., Mumbai, India. Another test chemical selected as chelating agent was Zeolite (Type-Y, Sodium form), obtained from Hi-Media Laboratories Ltd., Mumbai, India.

The experimental group of five fishes were exposed to four different compositions of test solution, *i.e.* two doses (200 mL & 400 mL) of 1% solution of sodium arsenite in 20 L water and two doses (200 mL & 400 mL) of 1% solution of sodium arsenite along with 1% zeolite in 20 L water in a glass aquaria of size 76.2 cm x45.72 cm x 45.72 cm. The test

animals for all set of experiments were exposed with both test solutions for 3days, 7days and 15 days for evaluation of acute toxicity. The water of aquaria was changed daily for complete duration of experiment and physico-chemical parameters were maintained. For evaluation of toxicity by arsenic and chelating effect of zeolite, the parameters – GPT, GOT and ALP were selected.

After exposure to specific dose, all fishes were sacrificed. Liver tissue was taken out in test tubes, kept in ice cold water. The tissues were then homogenized using a glass homogenizer with 5% trypsin. The homogenate was filtered through Watman No. 1 filter paper and then used for testing enzymatic activities. Estimation of activities of GOT & GPT was done following Reitman and Frankel's method (1957) by End point reaction and ALP was estimated by visible kinetic reaction (Bowers &McComb, 1966). Statistical analysis of the results was done by two way ANOVA, using the software Minitab.

RESULT

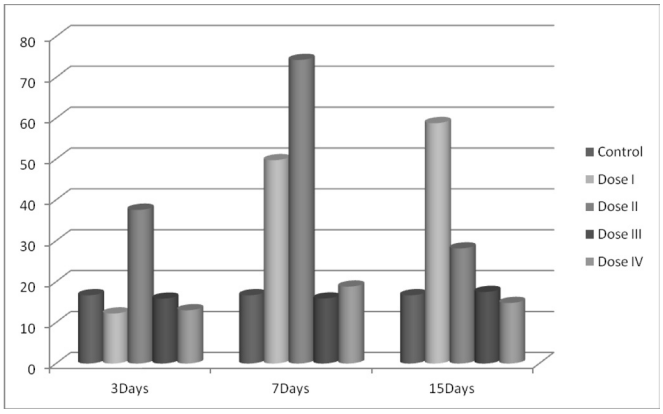
The toxic effects of arsenic on GPT, GOT and Alkaline phosphatise activity of *Heteropneustes fossilis* exposed to different doses of sodium arsenite and the chelating effect of zeolite were examined and the results were as below.

Alteration in GPT activity

The control value of SGPT activity from liver extract was found to be16.69±3.39 UL^{-1} and it was found decreased up to 12.22±0.81 UL^{-1} after exposure of 200 mL of 1% sodium arsenite for 3days, but after exposure of 400 mL 1% sodium arsenite for same duration it was found increased up to 37.58±18.18 UL^{-1}. For 7 days exposure, the activity of SGPT was found increased up to 49.75±2.95 UL^{-1} in response of 200 mL of sodium arsenite and up to 124.25±3.18 UL^{-1} in response of 400 mL sodium arsenite. Similarly for 15 days exposure, it was found increased up to 58.80±5.79 UL^{-1} in response of 200 mL and up to 28.14±0.22 UL^{-1} in response of exposure to 400 mL sodium arsenite.

After chelation with zeolite, the value of GPT activity reported for 3days duration was 15.86±1.71 UL^{-1} for 200 mL and 13.01±0.95UL^{-1} for 400 mL of the solution (sodium arsenite + zeolite). For 7days exposure, the activity reported was 15.89±3.18UL^{-1} and 18.81±1.89 UL^{-1}, respectively on exposure to 200 mL and 400 mL of the solution. For 15 days exposure, the activity reported was 17.50±1.61UL^{-1} and 14.80±1.32 UL^{-1}, respectively for 200 mL and 400 mL of the solution.

The activity of GPT in liver of *Hetropneustes fossilis* exposed to sodium arsenite was found increased significantly ($p<0.05$), after exposure to doses, 200 mL and 400 mL, for 7 days and 15 days duration each, in comparison to control group. But the activity of the enzyme decreased significantly ($p<0.05$) to normal range compared to control, after treatment with zeolite, for all exposures.



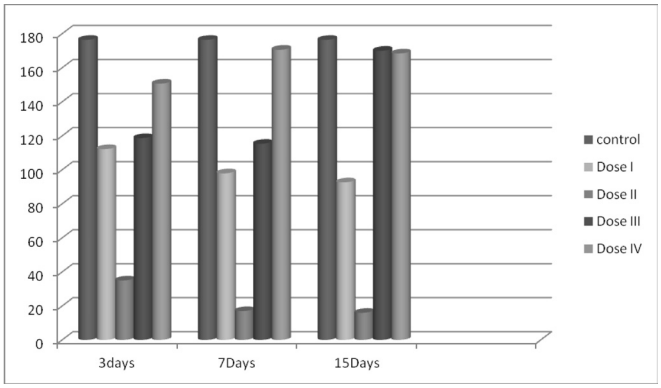
Dose I: Sod.arsenite (200ml); Dose II:Sod.arsenite (400ml); Dose III:Sod. arsenite+Zeolite(200ml); Dose IV:Sod.arsenite+Zeolite(400ml).

Figure 2 - Figure showing activity of GPT from liver of *Heteropneustes fossilis* on exposure to different concentrations of sodium arsenite and sodium arsenite + zeolite.

Alteration in GOT activity

The control value of GOT activity from liver extract was found to be 176.40±11.27 UL^{-1} and it was found decreased to 12.22±1.56 UL^{-1} after exposure of 200 mL of 1%sodium arsenite for 3 days and 35.00±9.56 UL^{-1} after exposure of 400 mL of 1% sodium arsenite for the same duration. For seven days exposure, the activity was reported to be decreased to 27.94±5.45 UL^{-1} in response of 200 mL and up to 17.03±5.13 UL^{-1}in response of 400 mL. Similarly for 15 days exposure, it was reported decreased to 92.69±4.61UL^{-1} in response of 200 mL and to 15.99±2.74UL^{-1} in response of 400 mL of sodium arsenite.

After chelation with zeolite, the value of GOT activity reported for 3days duration was 118.70±9.39 UL^{-1} for 200 mL and 150.66±11.30 UL^{-1} for 400 mL of the solution (sodium arsenite + zeolite). For 7days exposure, the activity reported was 115.36±3.6 UL^{-1} and 170.60±8.41UL^{-1}, respectively for 200 mL and 400 mL of the solution. For 15 days exposure, the activity reported was 170.00±9.23UL^{-1} and 168.47±8.31 UL^{-1}, respectively for 200 mL and 400 mL of the solution.



Dose I: Sod.arsenite (200ml); Dose II:Sod.arsenite (400ml); Dose III:Sod. arsenite+Zeolite(200ml); Dose IV:Sod.arsenite+Zeolite(400ml).

Figure 3 - Figure showing activity of GOT from liver of *Heteropneustes fossilis* on exposure to different concentrations of sodium arsenite and sodium arsenite + zeolite.

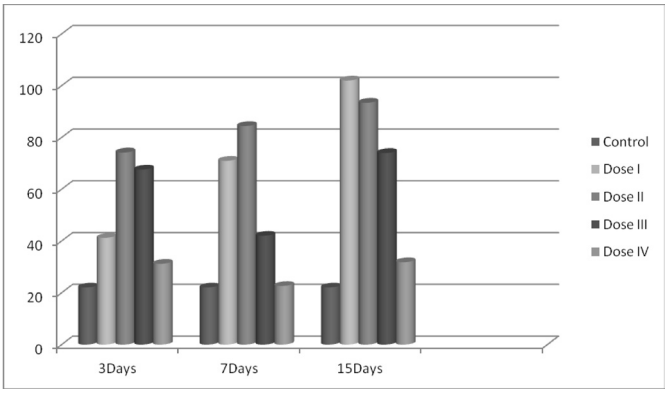
The activity of GOT showed a significant decrease ($p<0.05$) from control value on exposure to all doses of exposure to sodium arsenite. After treatment with zeolite along with sodium arsenite, an increase in the activity of GOT ($p<0.05$) was observed, in comparison to control, especially with longer duration and higher concentration of the test solution.

Alteration in Alkaline phosphatase activity

Similarly, the control value for activity of Alkaline phosphatase from liver extract obtained was 22.00±3.94 UL^{-1} and it was found increased to 41.20±7.56 UL^{-1} after exposure of 200 mL of 1% sodium arsenite for 3 days and 74.20±6.65 UL^{-1} after exposure of 400 mL of 1% sodium arsenite for the same duration. For seven days exposure, the activity was reported to be increased to 71.00±3.61UL^{-1} in response of 200 mL and up to 84.40±10.14 UL^{-1} in response of 400 mL of sodium arsenite. Similarly for 15 days exposure, it was reported increased to 102.00±8.94 UL^{-1} in response of 200 mL and to 93.40±40.35 UL^{-1} in response of 400 mL of sodium arsenite.

After chelation with zeolite, the value of activity of Alkaline phosphatase reported for 3days duration was 67.60±13.88 UL^{-1} for 200 mL and 31.20±1.30 UL^{-1} for 400 mL of the solution (sodium arsenite + zeolite). For 7days exposure, the activity reported was 42.00±3.17 UL^{-1} and 22.60±11.44 UL^{-1}, respectively for 200 mL and 400 mL of the solution. For 15 days exposure, the activity reported was 74.00±1.58 UL^{-1} and 31.80±1.19 UL^{-1}, respectively for 200 mL and 400 mL of the solution.

The activity of Alkaline phosphatase showed a significant increase ($p<0.05$) for all set of exposures of the test chemical (sodium arsenite). On treatment with zeolite, the activity of the enzyme decreased significantly, for all set of exposures, in comparison to control ($p<0.05$).In this experiment also it was observed that the decrease in the activity of the enzyme was more prominent on exposure to longer duration and higher concentration of the chelating agent.



Dose I: Sod.arsenite (200ml); Dose II:Sod.arsenite (400ml); Dose III:Sod. arsenite+Zeolite(200ml); Dose IV:Sod.arsenite+Zeolite(400ml).

Figure 4 - Figure showing activity of Alkaline phosphatase from liver of *Heteropneustes fossilis* on exposure to different concentrations of sodium arsenite and sodium arsenite + zeolite.

The activity of GPT in liver of *Hetropneustes fossilis* exposed to sodium arsenite was found increased significantly (F=46.63>14.24),at 5% P, after exposure to doses, 200 mL and 400 mL, for 7 days and 15 days duration, in comparison to control group. But the activity of the enzyme decreased significantly (F=47.78>14.24) to normal range compared to control, after treatment with zeolite, for all exposures.

The activity of GOT showed a significant decrease (F=132.12>49.69) from control value on exposure to sodium arsenite of all doses. But an increase in the activity of GOT (107.21>49.69) was observed when the fishes were treated with zeolite.

At the same time, the activity of Alkaline phosphatase showed a significant increase (F=595>190.97) for all set of exposures of the test chemical to sodium arsenite. On treatment with zeolite, the activity of the enzyme decreased significantly, for all set of exposures, in comparison to control (562.33>190.97) at 5% P.

DISCUSSION

In the experiments sodium arsenite was observed to cause significant ($P<0.05$) increase in the activity of GPT & Alkaline phosphatase and decrease in the activity of GOT in the liver. These results suggested the inhibitory role played by arsenite in cellular metabolism. Addition of zeolite to arsenic contaminated media, significantly reduced ($P<0.05$) the metal level in water and making it less available to the animal, which in turn helped to improve the enzyme activity.

Several authors have reported increased activity of GPT and GOT in various animals under arsenic intoxication. Humtsoe *et al*., in 2007 have studied the effect of sodium arsenicum ($Na_2HAsO_4.7H_2O$) (with 96 and 144 mgL^{-1} sub lethal concentration), on different enzymes of juvenile *Labeo rohita*, acclimatized in glass aquarium (36x24x24 cm) with constant physic-chemical conditions (oxygen-6.00-6.50mgL^{-1}, pH 7.2-7.5 and temperature 27-29°C). After one month exposure they analysed activity of GOT, GPT, ACP and ALP and found significantly decreased activities for all enzymes at 144 mg L^{-1} concentration, but the activity of ACP and ALP was found profoundly decreased. The findings of Humtsoe *et al.* was affirmative to our finding for GOT but our finding differ from their finding for ALP and GPT.

In arsenic treated rabbit SGPT and SGOT activity was reported significantly increased by Ahmad in 2004. Islam *et al*., (2005) have reported significant increase in SGPT and SGOT activity in arsenic treated rat. In arsenic treated Swiss albino mice increased activity of SGPT, SGOT, ALP and ACP was also reported by Sharma *et al.* in 2007. Our findings were affirmative to the findings of the above scientists regarding the activity of ALP and GPT.

Roy in 2002 reported that the liver is major organ for removal of xenobiotics in fishes. Karatas and Kalay in 2002 have also attributed that different organic and inorganic chemicals adversely affects integrity of hepatic cell

organelles and membrane transports which leads to alteration in metabolic pathways.

In 2007, Vutukuru *et al*., have also reported imbalance in hepatic enzyme activity in arsenic exposed *Labeo rohita*. They conducted experiment to evaluate toxic effect of both arsenic and hexavalent chromium on enzymatic activity of *Labeo rohita* and reported that there was a significant increase in the activity of hepatic enzyme ALT, however no such significant change was observed in chromium exposed fish and concluded ALT as biomarker for arsenic induced hepatotoxicity in *Labeo rohita.*

In present study changed enzymatic activity indicates damage to hepatic cells under intoxication of arsenic. It is also an established fact that arsenic toxicity causes oxidative stress and antioxidant enzymes like glutathione dependant enzymes act positively against arsenic for defence. A decrease in glutathione-s-transferase, glutathione peroxidise, glutathione reductase and catalase was observed by Allen and Rana in 2004 in arsenic exposed liver and kidney. This can be co-relative with changed activity of GOT and GPT in test animal exposed to arsenic.

Jain *et al*., in 1999 have studied the protective role of zeolite in lead induced toxicity in *Heteropneustes fossilis*. In an experiment they found that 35 and 120 days exposure of sublethal doses of lead nitrate to *Heteropneustes fossilis* resulted in decrease in soluble protein, RNA and glycogen in liver and increase in serum cholesterol but the presence of zeolite in exposure solution decreased all the adverse effects. Although, literature related to arsenic removal by zeolite is not available but affinity of zeolite towards many toxic cations based on ion exchange with respect to precipitation was reported by several authors (Pansini *et al*., 1989: Jain *et al*., 1999).

Halimoon & Yin (2010) reported that removal of heavy metals from textile waste water was effective using zeolite, Erdem *et al.,* (2004) also reported that natural zeolites can be used effectively for the removal of metal cations from wastewater. Shevade & Ford (2004) proved that NY6 zeolites were very effective for arsenic removal from polluted water.

Our finding is comparable with the findings observed in literature with regards to the alteration in activity of GPT, GOT and Alkaline phosphatase in animals exposed to arsenic and other heavy metals. The findings of the present study indicate that arsenic exposure is responsible for significant alteration in activity of GPT, GOT and Alkaline phosphatase in comparison to control in *Heteropneustes fossilis* and treatment with zeolite could significantly bring recovery of the conditions in the fish.

The efficacy of zeolites in reducing heavy metals, other than arsenic from animal body was evaluated by many studies. But chelating effect of zeolite for arsenic toxicity has not been properly examined. No literature is available for zeolite based chelation of arsenic from any animal body, that is why an attempt was made and significant result was found. We can conclude that like other heavy metals, arsenic toxicity may also be reduced from aquatic fauna by using zeolite. Further structural specification of zeolite and related efficacy of chelation of arsenic is yet to be established.

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