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ORIGINAL ARTICLE

Study of Arsenic Induced Alteration in Renal Function in *Heteropneustes fossilis*, and It's Chelation by Zeolite

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ABSTRACT
Arsenic is a heavy metal toxicant that occurs naturally in water, soil and air. As a result of natural and anthropogenic process it is widespread in the environment. In the current study, an attempt has been made to analyse the arsenic induced biochemical changes related to kidney functioning in Heteropneustes fossilis. Fishes were exposed to two different concentrations of sodium arsenite (200ml and 400ml of 1% solution), for 3 different durations (3days, 7days and 15 days).The alterations in concentration of creatinine and urea from blood and liver extract were analyzed. The concentrations of total Creatinine (F=15.99 > 3.84 at 5% P in liver; F=6.09 > 3.84 at 5% P in blood), and Urea (F=1.56 < 3.84 at 5% P in liver; F=0.82< 3.84 at 5% P in blood) were found increased along with increasing concentration and duration of sodium arsenite. The toxic effect was found recovered after application of synthetic zeolite for all parameters, i.e., Creatinine (F=1.12>0.68 in liver; F= 0.78>0.68 in blood) and Urea (F=132.12>49.69 at 5% P in blood; statistically insignificant in liver). The results suggest that, zeolite is a potential compound for decreasing significantly the load of toxicity of arsenic in aquatic fauna.
Key words: Sodium arsenite, synthetic zeolite, Heteropneustes fossilis, creatinine, urea, adsorption

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INTRODUCTION

Arsenic toxicity is a global health problem affecting many millions of people. Unlike other chemical contaminants that are found in limited locations and point sources, high levels of arsenic have been identified in many water supplies around the world. In some affected areas, such as India, Bangladesh, Taiwan and possibly China, the sizes of exposed populations are very large. Globally, many millions of people drink water containing unacceptably high arsenic levels, causing many health problems [1].The objective of the present work is to study the effect of sodium arsenite on renal functions in *Heteropneustes fossilis* and to evaluate the efficacy of zeolite in its chelation.

Symptoms of arsenicosis are primarily manifested in the forms of different types of skin disorder such as skin lesions, hyperkeratosis and melanosis [2].Arsenic is classified as a group A and category 1 human carcinogen by the USEPA [3] and the international association of research on cancer [4] respectively. Pandey *et al*., [5] first reported arsenic contamination in human in village named Kaurikasa in Chhattisgarh.

Arsenic is one of the most toxic elements that can be found. Humans may be exposed to arsenic through food, water and air. Exposure may also occur through skin contact with soil or water that contains arsenic. Levels of arsenic in fish and seafood may be high, because fish absorb arsenic from the water they live in. Luckily this is mainly the fairly harmless organic form of arsenic, but fish that contain significant amounts of inorganic arsenic may be a danger to human health.

Fishes that absorb considerable amount of arsenic are dangerous to human health as the chemical enter human body through food chain. No treatment of proven benefit is currently available to treat chronic arsenic toxicity. Treatment options advocated are vitamin and mineral supplements and antioxidant therapy. The benefits of these treatment measures need to be evidence based to receive endorsement and wider application. The efficacy of zeolites, both natural and synthetic, in removal of toxic heavy metals from environment has been proven by many researchers. Some workers have studied about the chelating property of zeolites against many heavy metals from living systems. In our present work an effort to

evaluate the efficiency of Synthetic zeolite in arsenic chelation from fishes' body was undertaken and the results were positive.

Zeolites are crystalline solid structure made of silicon, aluminium and oxygen that form a framework with cavities and channels inside where cations, water and/or small molecules may reside. They are microporous and hence often referred to as molecular sieves and are used as commercial adsorbents. Many of them occur naturally as minerals, and are extensively mined in many parts of the world and have applications in industry and medicine. However most of the Zeolites have been made synthetically, some of them for commercial use, while others created by scientists to study their chemistry [6].

In the past, Zeolites have been widely used in water and air filtration to remove toxins. They are also used in soil maintenance to purify the soil. Animal feed often has zeolites added. The Russians even used Zeolite powder at Chernobyl to soak up excess radiation. Zeolites have many other useful purposes. They can perform ion exchange, filtering, odour removal, and chemical sieve and gas absorption tasks. The most well-known use for zeolites is in water filtration applications.

Synthetic zeolites hold some key advantages over their natural analogues. The synthetics can, of course, be manufactured in a uniform, phase-pure state. It is also possible to manufacture desirable zeolite structures which do not appear in nature. Synthetic zeolites hold some key advantages over their natural analogues. The synthetics can, of course, be manufactured in a uniform, phase-pure state. It is also possible to manufacture desirable zeolite structures which do not appear in nature.

Faujasite is popular synthetic zeolite and the most commonly found faujasites are zeolites X (higherAl) and zeolite Y (lower Al). Zeolite Y is the most important catalytic zeolite, and is generally synthesized in the Na form [7].We have chosen synthetic zeolite Y (Sodium form) for the present study to evaluate it efficacy in chelation of arsenic from fish body.

MATERIALS AND METHODS

The Teleost cat fish, *Heteropneustes fossilis*, was selected for experiment and were collected from a local pond. The fish selected for experiment was 7-8 inch of length and average weight of 125-150 gms. and were maintained in glass aquarium of 2 ½ feet x1 ½ feet x 1 ½ feet dimension, with 20L of chlorine-free bore well water. The fishes selected for experiment were first acclimatized in the lab. The fishes were then divided into five experimental groups, each with 5 fishes. The first group of fishes was selected for control sets and next four for experimental set, 200ml sodium arsenite in 20L water, 400ml sodium arsenite in 20L water, 200ml sodium arsenite in 20L water with zeolite and 400ml sodium arsenite in 20L water with zeolite.

The test chemical selected for the experiment was arsenic trioxide obtained from s.d. Fine – Chem Ltd, Mumbai. The experimental dose of 200 ml of 1% sodium arsenite was prepared for 20 L water by dissolving 100mg of arsenic trioxide per 100ml of aqueous solution of sodium hydroxide, to make the stock solution. A similar dose of 400 ml (1%) for 20 L of water was also prepared. Another test chemical selected as chelating agent was Zeolite (Type-Y, Sodium form), obtained from Hi-Media Laboratories Ltd., Bombay. A combination of Zeolite (1%) with 200ml and 400ml of sodium arsenite solution was prepared for the experiment. For all sets of experiments, three durations of exposures were selected, i. e, 3days, 7days and 15days for evaluation of acute toxicity.

After exposure for specific dose and duration, blood was collected in a glass vial pre-coated with anticoagulant (EDTA) by the help of glass syringe from caudal vein of each fish. Liver tissues were taken out and kept in test tubes and placed in a container with ice cubes, and then homogenized in a homogenizer with 5% trypsin. The homogenate was filtered before biochemical analysis. Estimation of urea was done by U V kinetic method [8] and creatinine by two point reaction [9] method. Statistical analysis of the results was done by two way ANOVA.

RESULT

Alteration in creatinine

In our observation (Ref: Table-1) the average control value for creatinine from liver was found to be 1.94±0.41 mg/dl. After exposure to 200ml of sodium arsenite for three days, it was found increased up to 2.2±0.34mg/dl, for 7 days exposure it was 2.80±0.19mg/dl, and for 15 days exposure of the same dose, the concentration of creatinine obtained was 2.12±0.13mg/dl. When exposed to 400ml of the test solution for three days, the average amount of total cholesterol was increased to 2.86±0.19mg /dl, for 7 days exposure the average concentration was 2.22±0.11mg/dl and for 15 days exposure the value was 3.11±0.15mg/dl. But when exposed to 200ml solution of zeolite along with sodium arsenite the average concentration obtained was decreased to 0.47±0.10mg/dl for 3days, 0.73±0.04mg/dl for 7days and 0.36±0.05mg/dl for 15 days exposure. Similarly when exposed to 400ml of sodium arsenite with zeolite,

average concentration measured was 1.26±0.29mg/dl for 3days exposure; 0.14±0.05mg/dl for 7days exposure; 1.08±0.20mg/dl for 15 days exposure.

The average control value for creatinine from blood was found to be 0.80±0.01 mg/dl. After exposure to 200ml of sodium arsenite for three days, it was found increased up to1.20±0.92mg/dl, for 7 days exposure it was 1.17±0.19mg/dl, and for 15 days exposure of the same dose, the concentration of creatinine obtained was 1.65±0.33mg/dl. When exposed to 400ml of the test solution for three days, the average amount of total cholesterol was increased to 1.30±0.13mg /dl, for 7 days exposure the average concentration was 1.47±0.09mg/dl and for 15 days exposure the value was 0.89±0.08mg/dl. But when exposed to 200ml solution of zeolite along with sodium arsenite the average concentration obtained was decreased to 0.47±0.10mg/dl for 3days, 0.73±0.04mg/dl for 7days and 0.36±0.05mg/dl for 15 days exposure. Similarly when exposed to 400ml of sodium arsenite with zeolite, average concentration measured was 0.80±0.29mg/dl for 3days exposure; 0.14±0.05mg/dl for 7days exposure; 0.54±0.03mg/dl for 15 days exposure.

The concentration of creatinine from liver and blood of *Hetropneustes fossilis* exposed to sodium arsenite was found increased significantly ($F=15.99 > 3.84$ at 5% P in liver; $F=6.09 > 3.84$ at 5% P in blood), after exposure to doses, 200ml and 400ml, for all durations, in comparison to control group. But the value decreased significantly to normal range, compared to control, after treatment with zeolite ($F=1.12>0.68$ in liver; $F= 0.78>0.68$ in blood), for all exposures. This is an indication of the chelating effect of zeolite on the toxicant.

Alteration in urea

The average control value for urea from liver was found to be 4.66±1.98 mg/dl. After exposure to 200ml of sodium arsenite for three days, it was found increased up to3.20±0.60mg/dl, for 7 days exposure it was 8.72±3.72mg/dl, and for 15 days exposure of the same dose, the concentration of urea obtained was 79.58±4.6mg/dl. When exposed to 400ml of the test solution for three days, the average amount of total cholesterol was increased to 8.26±4.48mg /dl, for 7 days exposure the average concentration was 9.49±1.00mg/dl and for 15 days exposure the value was 42.21±1.77mg/dl. But when exposed to 200ml solution of zeolite along with sodium arsenite the average concentration obtained was decreased to 6.42±4.79mg/dl for 3days, 2.12±0.39mg/dl for 7days and 4.66±1.22mg/dl for 15 days exposure. Similarly when exposed to 400ml of sodium arsenite with zeolite, average concentration measured was 0.90±0.03mg/dl for 3days exposure; 4.98±0.68mg/dl for 7days exposure; 5.44±0.69mg/dl for 15 days exposure.

The average control value for urea from blood was found to be 6.22±0.28 mg/dl. After exposure to 200ml of sodium arsenite for three days, it was found increased up to3.20±0.29mg/dl, for 7 days exposure it was 8.72±0.44mg/dl, and for 15 days exposure of the same dose, the concentration of urea obtained was 79.58±4.0mg/dl. When exposed to 400ml of the test solution for three days, the average amount of total cholesterol was increased to 13.85±0.48mg /dl, for 7 days exposure the average concentration was 9.48±2.15mg/dl and for 15 days exposure the value was 8.00±0.21mg/dl. But when exposed to 200ml solution of zeolite along with sodium arsenite the average concentration obtained was decreased to 3.60±0.50mg/dl for 3days, 5.50±0.21mg/dl for 7days and 4.66±0.42mg/dl for 15 days exposure. Similarly when exposed to 400ml of sodium arsenite with zeolite, average concentration measured was 4.50±0.97mg/dl for 3days exposure; 4.98±0.48mg/dl for 7days exposure; 1.15±0.03mg/dl for 15 days exposure.

The concentration of urea from liver and blood of *Hetropneustes fossilis* exposed to sodium arsenite was found increased (but statistically found to be non-significant), after exposure to doses, 200ml and 400ml, for all durations, in comparison to control group. But the value decreased significantly to normal range, compared to control, after treatment with zeolite ($F=132.12>49.69$ at 5% P in blood; statistically non-significant in liver), for all exposures. This is an indication of the chelating effect of zeolite on the toxicant.

DISCUSSION

Our study reports significantly elevated level ($p<0.05$) of creatinine and urea after exposure to sodium arsenite and significant reduction of the value after exposure to zeolite. There are relatively few clinical reports available on arsenic induced nephrotoxicity in animals. Saxena *et al*., [10] reported significant increase in serum creatinine and urea in arsenic treated albino rats. Nephrotoxicity was assessed by estimating the serum levels of urea, uric acid and creatinine, the markers of renal dysfunctioning. Arsenic trioxide intoxication significantly increased the serum level of urea, uric acid and creatinine in comparison to control due to renal dysfunctioning, as per the authors.

Patel and Kalia[11] reported increased blood urea and serum creatinine in experimental diabetic rats after subchronic exposure to arsenic. Missoun *et al.,* [12] reported significant increase in serum creatinine and urea levels in Wistar rats treated to the heavy metal lead. Renal function impairment might result

from intrinsic renal lesions, decreased perfusion of the kidney or due to deranged metabolic process caused by metal toxicity[13].Exposure to arsenic or its various forms can lead to induce nephrotoxicity in experimental animals [14].High concentration of arsenic can accumulate in the kidney tissue than other tissues through various exposure routes [15].

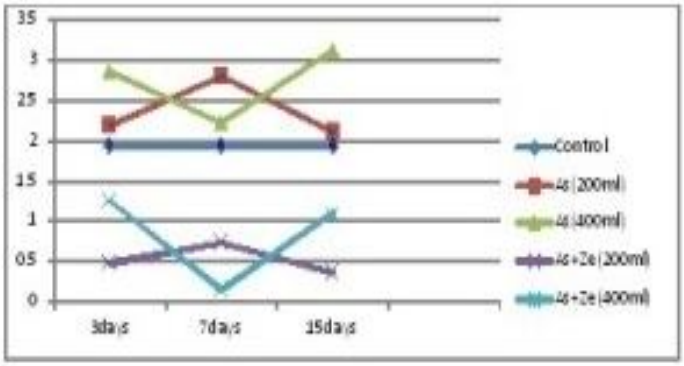

Fig:1:- Alteration in creatinine from liver extract of *Heteropneustes fossilis* after acute exposure of sodium arsenite and sodium arsenite + zeolite. (Values in gm/dl)

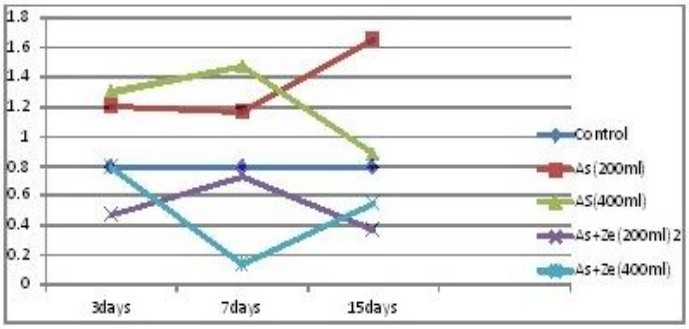

Fig:2:- Alteration in creatinine from blood extract of *Heteropneustes fossilis* after acute exposure of sodium arsenite and sodium arsenite + zeolite. (Values in gm/dl)

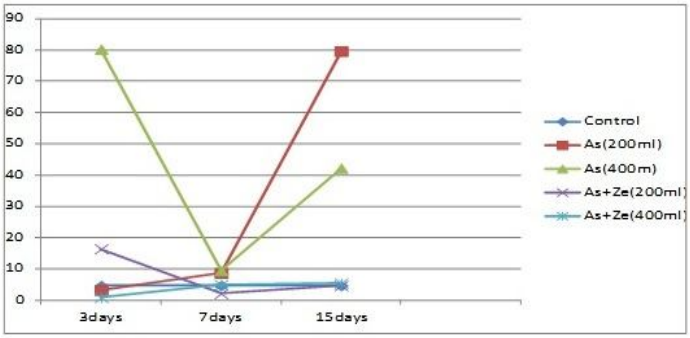

Fig:3:- Alteration in urea from liver extract of *Heteropneustes fossilis* after acute exposure of sodium arsenite and sodium arsenite + zeolite. (Values in gm/dl)

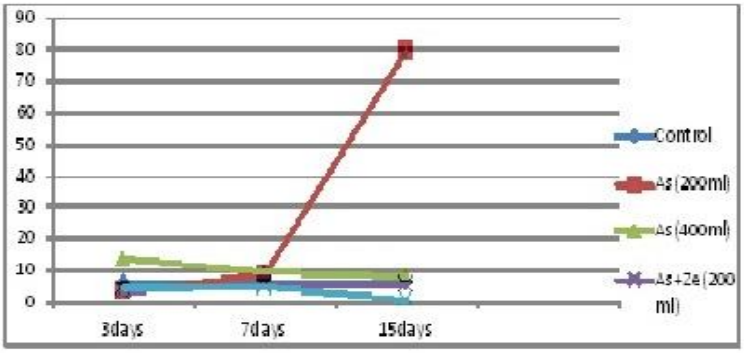

Fig:4:- Alteration in urea from blood extract of *Heteropneustes fossilis* after acute exposure of sodium arsenite and sodium arsenite + zeolite. (Values in gm/dl)

Table:1:-Table showing alterations in concentration of creatinine and urea in liver and blood of *Heteropneustes fossilis* exposed to sodium arsenite and sodium arsenite with zeolite.

Days	Groups	Creatinine from liver	Creatinine from blood	Urea fromliver	Urea from blood
3 days	Control	1.94±0.41	0.80±0.01	4.66±1.98	6.22±0.28
	Arsenic-I dose	2.2±0.34	1.20±0.92	3.20±0.60	3.20±0.29
	Arsenic-IIdose	2.86±0.19	1.30±0.13	8.26±4.48	13.85±0.5
	Arsenic+Zeolite-Idose	0.47±0.10	0.47±0.10	6.42±4.79	3.60±0.50
	Arsenic+Zeolite-IIDose	1.26±0.29	0.80±0.29	0.90±0.03	4.50±0.97
7 days	Control	1.94±0.41	0.80±0.01	4.66±1.98	6.22±0.28
	Arsenic-I dose	2.80±0.19	1.17±0.19	8.72±3.72	8.72±0.44
	Arsenic-IIdose	2.22±0.11	1.47±0.09	9.49±1.00	9.48±2.15
	Arsenic+Zeolite-Idose	0.73±0.04	0.73±0.04	2.12±0.39	5.50±0.21
	Arsenic+Zeolite-IIDose	0.14±0.05	0.14±0.05	4.98±0.68	4.98±0.48
15days	Control	1.94±0.41	0.80±0.01	4.66±1.98	6.22±0.28
	Arsenic-I dose	2.12±0.13	1.65±0.33	79.58±4.6	79.58±0.4
	Arsenic-IIdose	3.11±0.15	0.89±0.08	42.21±1.8	8.00±0.21
	Arsenic+Zeolite-Idose	0.36±0.05	0.36±0.05	4.66±1.22	4.66±0.42
	Arsenic+Zeolite-IIDose	1.08±0.20	0.54±0.03	5.44±0.69	1.15±0.03

Values in g/dl (Mean±SD)
I dose: 200ml of the test solution; II dose 400ml of the test solution

CONCLUSION

Our findings are comparable with the findings of the above scientists with regards to the alteration in creatinine and urea content 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 creatinine and urea from liver and blood 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 removing heavy metals, other than arsenic from animal body was evaluated by many workers. 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 load 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.

REFERENCES

1. Chakraborty. D., Samanta, G., Mandal, B.,K., Chaudhury, T.,R., Chanda., C.,R., Biswas, B.,K., Dhar, R.,K., Basu, G.,K., & Saha, K.,C., (1998): Calcutta's industrial pollution: Ground water Arsenic contamination in residential area and sufferings of people due to industrial effluents discharge-An eight-year study report. Current Science*:* 7 (4):346-360.
2. Tseng, W., P., (1977). Effects and dose response relationships of skin cancer and black foot disease with As. Environ. Health Perspect. 19: 109-119.
3. USEPA., (1997): IRIS (Integrated Risk Information System) on line data base maintained in Toxicology Data Network (TOXNET) by the National Library of Medicine, Bethesda, Maryland.
4. IARC., (International Agency for Research on Cancer) (1987). Arsenic and arsenic compounds, IARC monographs on the evaluation of carcinogenicity; updating of IARC monographs vol. 1-42. Lyon: IARC, 100 (suppl.7)
5. Pandey, P.,K., Khare, R.,N., Sharma, S., Sar, S.,K., Pandey, M., & Binayake, P., (1999). Arsenicosis and deteriorating groundwater quality: unfolding crisis in central east India region. *Curr Sci*. 77: 686-693.
6. Zeolites,AbbeyNewsletter,Volume20,Number7,Dec1996.(http:/www.asdn.net/aasdn/chemistry/zeolites.shtml)
7. Mravec, D., Hudec, J. & Janotka, I., (2003). Some possibilities of Catalytic and Non-Catalytic Utilization of Zeolites. Chem.Pap.59 (1)62-69.
8. Tiffany, T., O., Jausen, J., M., Burtis, C., A., Overtone, J.,B., & Scott, C.,D., (1972). Enzymatic zenetic rate and endpoint analyses of substrate by use of GEMSAEC festa analyser.Clin. chem.18:829-834.
9. Bowers, L.,D., Wong, E.,T., (1980) Kinetic serum creatinine assay II: A critical evaluation and review. Clin. Chem. 26: 555-561.
10. Saxena, P., N., Shalini A., Nishi S., & Priya B., (2009). Effect of arsenic trioxided on renal functions and its modulation by Cucurma aromatica leaf extract in albino rat. Journal of Environmental Biology, 30 (4), 527-531.
11. Patel, H.,V.,& Kalia,K., (2010); Sub chronic arsenic exposure aggravates nephrotoxicity in experimental diabetic rats. J. of Exp. Biology. Vol 48, pp.762-768.
12. Missoun, F., Slimani, M., & Aoues, A., (2010).Toxic effect of lead on kidney function in rat Wistar. African Journal of Biochemistry Research ,4(2), 21-27.
13. Cameron, J., S., & Greger, R., (1998). Renal function and testing of function. (Davidson AM, Cameron JS, Grunfeld

JP, Kerr DNS, Rits E, Winearl GC eds.) Oxford Textbook of Clinical Nephrology , 36-39.
14. Ayala-Fierro.F., Baldwin, A., C., Wilson, L. M., Valeski, J.,E., & Carter, D., E., (2007). Structural alterations of rat kidney after acute arsenic exposure. Lab Invest.80;87.
15. Kalia, K., Nurula, G, D., Kannan, G., M., &Flora, S., J., S., (2007). Effect of combined administration of Captopril and DMSA on arsenite induced oxidative stress and blood and tissue arsenic concentration in rats. Comp.Biochem.Physiol.C 144:327

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