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Arsenic Compound Induced Alteration in Protein Metabolism and Chelating Effect of Zeolite in *Heteropneustes fossilis*

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Abstract: ***The toxic effect caused by arsenic to humans and other organisms due to their occurrence in ground water and surface water is a serious health issue worldwide and many millions of people drink water containing unacceptably high arsenic levels. Industrial processes, mining, pesticides, fertilizers, paints and medicines can cause arsenic contamination. In the present work the effect of different concentrations of sodium arsenite in the liver of Heteropneustes fossilis, in terms of biochemical and histopathological aspects, and the chelating effect of zeolite was studied. Fishes were exposed to two different concentrations of sodium arsenite alone, and also two different concentrations of sodium arsenite along with zeolite for 3 different durations (3days, 7days and 15 days). Concentration of total protein, albumin and globulin from liver was estimated. A Significant decrease in total protein, albumin and globulin concentrations on exposure to sodium arsenite and recovery of the conditions, when the fishes were treated with zeolite along with sodium arsenite, was observed. The results of the histopathological study showed significant alterations in liver and zeolite treated arsenic did not cause any significant histopathological alterations in liver cells.***

Keywords: Sodium arsenite, Synthetic zeolite, *Heteropneustes fossilis*, protein metabolism, histopathology

1. Introduction

Arsenic is probably, the environmental contaminant that is responsible for the highest risk of mortality worldwide, mainly because of its toxicity and number of people affected. 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. Globally, many millions of people drink water that contained unacceptably high arsenic levels, causing many health problems. Arsenic contamination is caused mainly by the use of arsenic pesticides, industrial activities and mining operations [5].

Arsenicosis causes different types of skin disorder such as skin lesions, hyperkeratosis and melanosis [30, 31]. Arsenic is classified as a group A and category 1 human carcinogen by the USEPA, [32] and the international association of research on cancer [12] respectively. 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 [25, 30].

Exposure to inorganic arsenic can cause various health effects, such as irritation of the stomach and intestines, decreased production of red and white blood cells, skin changes and lung irritation. It is suggested that the uptake of significant amounts of inorganic arsenic can intensify the chances of cancer development, especially the chances of development of skin cancer, lung cancer, liver cancer and lymphatic cancer[3].

A very high exposure to inorganic arsenic can cause infertility and miscarriages with women, skin disturbances, declined resistance to infections, heart disruptions and brain damage in both men and women and can damage DNA. Studies have shown that arsenic can induce biochemical changes in the liver tissues of fish [19].

Zeolites are microporous materials made of aluminosilicates commonly used as commercial adsorbents. Zeolite is introduced in 1954 as adsorbent for industrial separations and purifications. It is well known for its ion exchange capacity. The role of zeolite in the conversion of solid and liquid hazardous wastes into environmentally acceptable products has also been demonstrated by Shevade *et al*. [23]. Several zeolites, namely clinoptilolite, chabazaite, SZP1, 13X and 5A have been identified as having potential for arsenic removal from water. Synthetic zeolites are useful because of their controlled and known physico-chemical properties relative to that for natural zeolites. It has been observed that the H+ and NH4 +forms of the synthetic zeolites are capable of removing arsenate to 50 ppb within 15 minutes , which is the current permitted maximum contaminant level (MCL) for arsenic in the United States [23]

It is considered as effective adsorbent because it can adsorb heavy metals from the wastewater sample .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 [7,13]

In the present study the effect of arsenic in causing protein metabolism alteration and histopathological alteration in *Heteropneustes fossilis* and the efficiency of synthetic zeolite, type Y, in removing arsenic toxicity was carried out.

2. 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 grams 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 laboratory. The fishes were then divided into five experimental groups, each with five. The first group of fishes was selected for control sets and next four for experimental set. The experimental sets were exposed to four different concentrations of the test chemicals as viz. 200ml of 1% solution of sodium arsenite, 400ml 1% solution of sodium arsenite, 200ml of 1% solution of sodium arsenite + zeolite and 400ml of 1% solution of sodium arsenite + zeolite, in 20L water. Each set of experiments were repeated for three different durations of exposures, ie, 3days, 7 days and 15 days.

The test chemical selected for the experiment was arsenic trioxide obtained from s.d. Fine –Chem Ltd, Mumbai, (India). A stock solution of 1% sodium arsenite was prepared by dissolving 1 gram arsenic trioxide per liter of 1% aqueous solution of sodium hydroxide. Another test chemical selected as chelating agent was Zeolite (Type-Y, Sodium form), obtained from Hi-Media Ltd. A combination of Zeolite (1%) with sodium arsenite solution was prepared for the experiment.

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 tissue was also taken out and homogenized for biochemical analysis. For homogenization, tissue were kept in the cube and then homogenized in a homogenizer with 5% trypsin. The homogenate was filtered before biochemical analysis. Estimation of total protein was done by Biuret method and Albumin and globulin by bromocresol green method [16]. The results obtained were statistically evaluated using two-way ANOVA.

For histopathological analysis, the liver tissue taken out and fixed in 10% formalin. The tissues were dehydrated in alcohol series and cleared in xylene. The tissues were embedded in paraffin wax which provides the necessary hardness to cut sections. When the paraffin was solidified, the tissue was made into blocks and cut into thin sections. The sections were then prepared for staining. To rehydrate the sections, they were floated into a microscope slide and then bonded to the slides by heating them above the melting point of the paraffin in an oven. The sections were then de waxed by immersing in xylene and hydrated by dipping in ethyl alcohol in decreasing gradients. The rehydrated sections were stained in Haematoxylene-Eosine stain and dehydrated again and then permanently mounted. The slides were observed under microscope and microphotographs were taken using Camera Lucida.

3. Results

Biochemical Alterations

We found that the concentration of total protein in liver decreased significantly ($p<0.05$) on exposure of 200ml and 400ml of 1% sodium arsenite solution in comparison to control group (Table 1; Fig. 1). The concentration of total protein reported from blood was also decreased significantly ($p<0.05$). But in groups treated with sodium arsenite along with zeolite, the value of total protein improved significantly ($p<0.05$) (Table 2, Fig.4).

The concentration of albumin altered significantly from control on exposure to sodium arsenite in liver as well as blood ($p<0.05$). The concentration decreased on exposure to 200ml of the chemical for the three durations and increased significantly on exposure to 400ml of the chemical. But treatment with solution of arsenite + zeolite brought the values back to normal in comparison with control (Table 1&2; Fig.2&5).

The globulin concentration reported from liver as well as blood was significantly ($p<0.05$) lowered in groups treated with arsenite alone, especially for 15 days duration, in comparison to control value. But the concentration increased in groups treated to solution of arsenite + zeolite (Table 1&2; Fig.3&6).

4. Tables

Table 1: Estimation of Protein from liver of Heteropneustes fossilis after acute exposure of sodium arsenite and sodium arsenite + zeolite.

Days	Groups	Total protein	Albumin	Globulin
3Days	Control	1.22±0.08	0.36±0.020.24±0.07	0.86±0.13
	Arsenic-I dose	0.28±0.09	0.93±0.11	0.04±0.01
	Arsenic-IIdose	1.10±0.46	0.38±0.05	0.17±0.02
	Arsenic+Zeolite-Idose	0.46±0.24	0.40±0.47	0.70±0.09
	Arsenic+Zeolite-IIDose	1.40±0.46		1.00±0.12
7Days	Control	1.22±0.08	0.36±0.02	0.86±0.13
	Arsenic-I dose	0.70±0.13	0.34±0.07	0.36±0.04
	Arsenic-IIdose	0.91±0.25	0.85±0.36	0.60±0.07
	Arsenic+Zeolite-Idose	0.94±0.5	0.36±00.40.44±0.08	0.58±0.10
	Arsenic+Zeolite-IIDose	1.92±0.04		1.48±0.12
15Days	Control	1.22±0.08	0.36±0.02	0.86±0.13
	Arsenic-I dose	0.20±0.01	0.16±0.03	0.40±0.02
	Arsenic-IIdose	0.95±0.12	0.90±0.07	0.50±0.04
	Arsenic+Zeolite-Idose	1.11±0.25	0.30±0.03	0.81±0.05
	Arsenic+Zeolite-IIDose	1.88±0.12	0.46±0.08	1.42±0.27

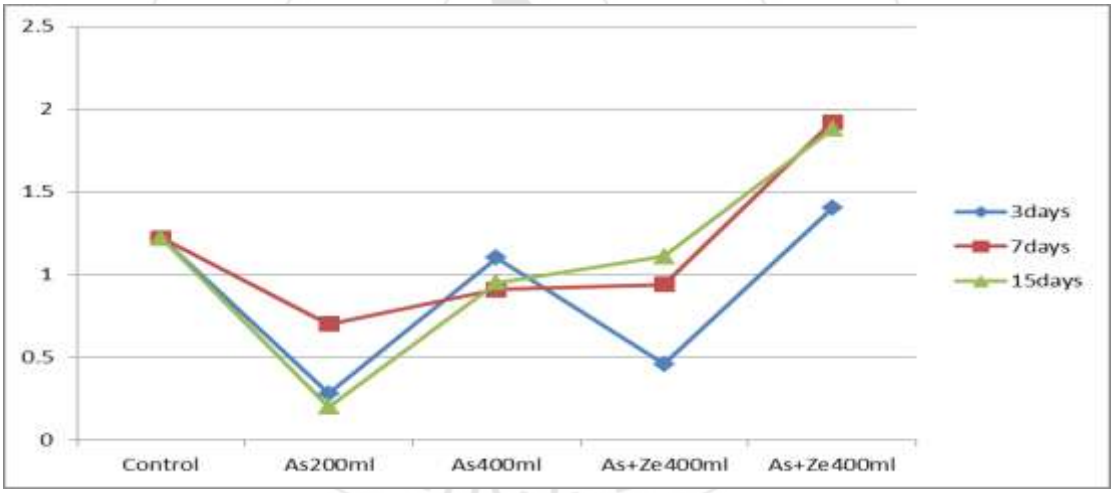
Values in gm/dl±SD
Arsenic: I Dose: 200ml II Dose: - 400ml
Arsenic+Zeolite: IDose: 200ml II Dose: - 400ml

Table 2: Estimation of Protein from blood of Heteropneustes fossilis after acute exposure of sodium arsenite and sodium arsenite + zeolite.

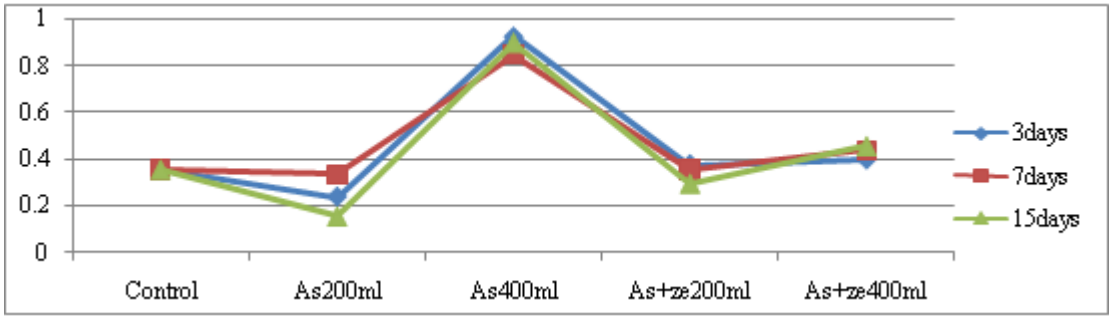
Days	Groups	Total protein	Albumin	Globulin
3Days	Control	3.54±0.33	2.55±0.33	0.99±0.13
	Arsenic-I dose	2.64±0.23	1.14±0.34	0.50±0.09
	Arsenic-IIdose	2.58±0.21	2.33±0.23	0.25±0.02
	Arsenic+Zeolite-Idose	1.48±0.10	0.80±0.23	0.68±0.03
	Arsenic+Zeolite-IIDose	3.13±0.06	2.14±0.18	0.99±0.05
7Days	Control	3.54±0.33	2.55±0.33	0.99±0.13
	Arsenic-I dose	2.13±0.22	1.60±0.30	0.53±0.03
	Arsenic-IIdose	1.57±0.04	0.53±0.04	1.04±0.02
	Arsenic+Zeolite-Idose	2.12±0.06	0.58±0.08	0.91±0.03
	Arsenic+Zeolite-IIDose	3.13±0.06	4.72±0.22	1.02±0.01
15Days	Control	3.54±0.33	2.55±0.33	0.99±0.13
	Arsenic-I dose	2.87±0.13	2.25±0.35	0.62±0.04
	Arsenic-IIdose	1.59±0.16	0.86±0.21	0.73±0.04
	Arsenic+Zeolite-Idose	3.76±0.18	2.56±0.23	1.20±0.04
	Arsenic+Zeolite-IIDose	4.65±0.5	4.50±0.68	1.15±0.07

Values in gm/dl±SD
Arsenic: I Dose: 200ml II Dose: - 400ml
Arsenic+Zeolite: IDose: 200ml II Dose: - 400ml

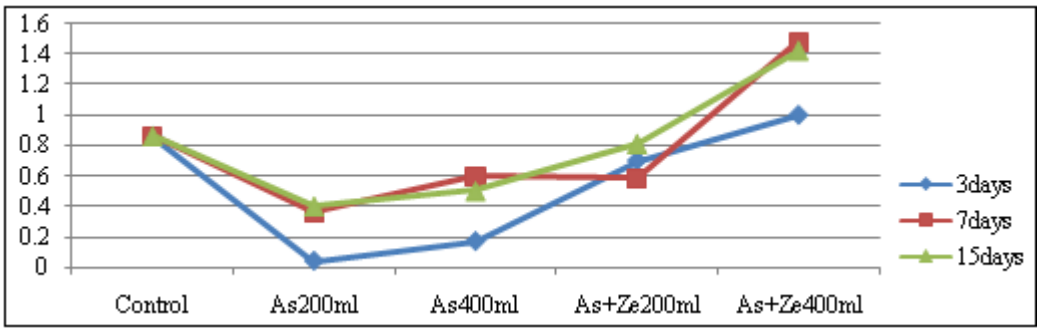
5. Figures



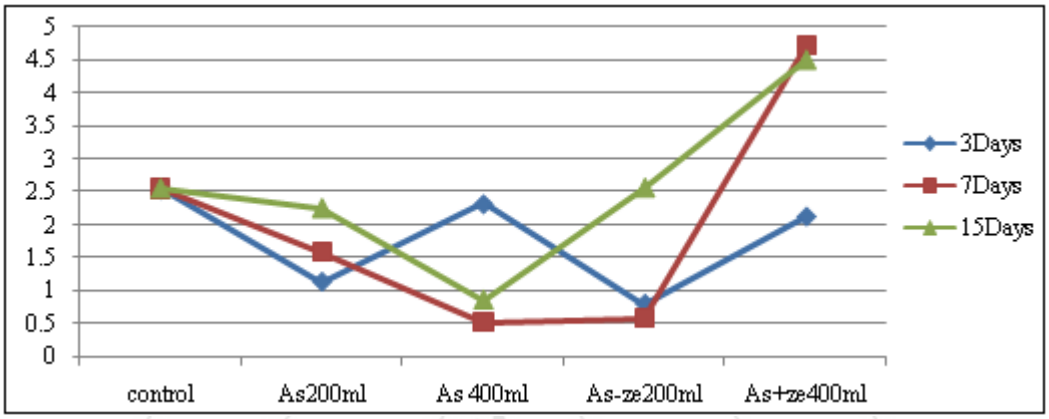
Figures 1: Alteration in Protein from liver of *Heteropneustes fossilis* after acute exposure of sodium arsenite and sodium arsenite + zeolite.



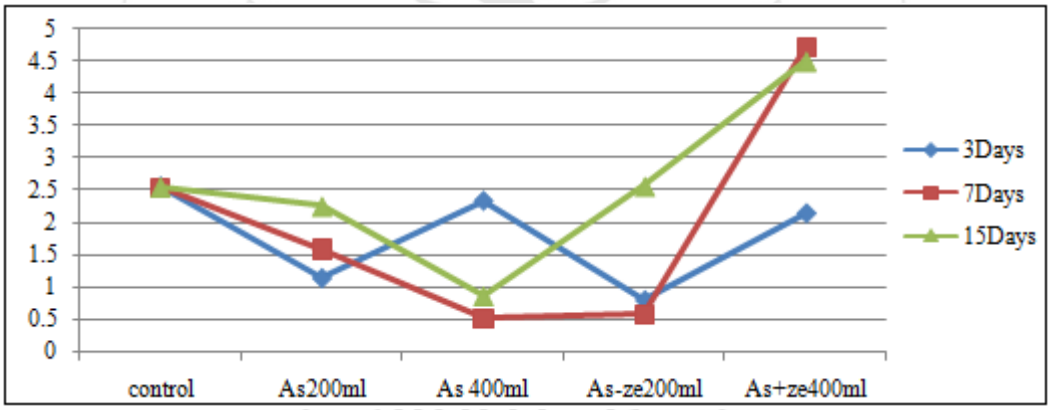
Figures 2: Alteration in Albumin from liver of *Heteropneustes fossilis* after acute exposure of sodium arsenite and sodium arsenite + zeolite.



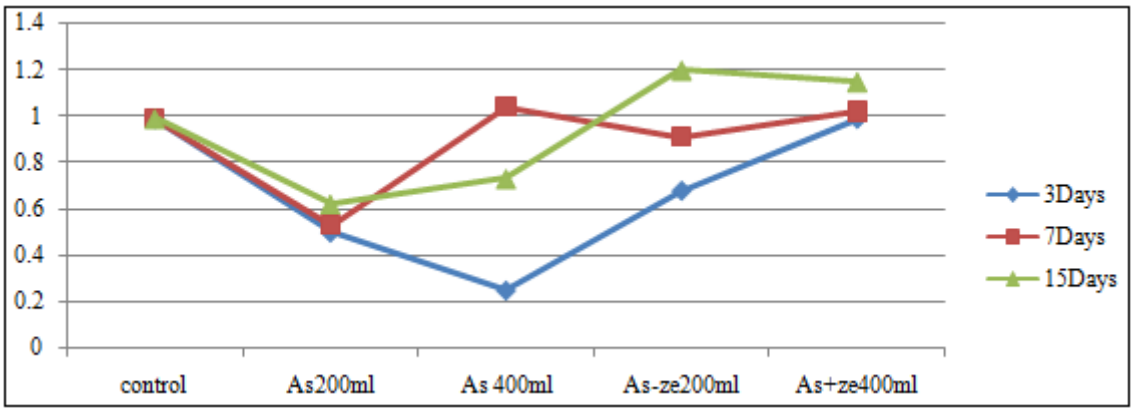
Figures 3: Alteration in Globulin from liver of *Heteropneustes fossilis* after acute exposure of sodium arsenite and sodium arsenite + zeolite



Figures 4: Alteration in Protein from blood of *Heteropneustes fossilis* after acute exposure of sodium arsenite and sodium arsenite + zeolite



Figures 5: Alteration in Albumin from blood of *Heteropneustes fossilis* after acute exposure of sodium arsenite and sodium arsenite + zeolite



Figures 6: Alteration in Globulin from blood of *Heteropneustes fossilis* after acute exposure of sodium arsenite and sodium arsenite + zeolite

6. Histopathological Alterations

The results of the studies on the effect of arsenic toxicity on histology of liver of *Heteropneustes fossilis* and its remediation using zeolite following exposure to different doses of sodium arsenite and sodium arsenite and zeolite are as follows.

In our study major histopathological alterations in liver was found as swelling (Ballooning), necrosis, cholestasis and hemosiderin formation besides mallory body formation. The hepatic necrosis and other related histopathological alterations may be due to oxidative stress induced by arsenic that further resulted in cellular protein degradation. Zeolite treated arsenic did not cause any significant histopathological alterations in liver cells in the above experiments.

After exposure to 200ml of 1% sodium arsenite solution for 3days some cells showed swelling (ballooning BL), due to accumulation of water in the cell probably due to proteins that normally are exposed. After 7days and 15 days exposures to the same concentration more inflammation in hepatocyte along with Swelling (BL) and mallary body (scattered hepatocytes-MB) formation were very apparent, beside necrosis (N) in cells were also very clear. After exposure to 400ml of 1% sodium arsenite solution for different duration, the cells showed not only ballooning (BL) and necrosis, but also cholestasis and hemosiderins (H) at several points, the intensity of which were more for longer durations.

On exposure to 200ml of 1% solution of sodium arsenite and zeolite for 3days, the cells showed inflammation (I), necrosis and ballooning at several points. On exposure to the same concentration for 7days, hepatocytes showed disaggregation and necrosis at some regions. But ballooning was not found at all. On 15 days exposure, almost normal histological texture was observed. Ballooning, necrosis, inflammation etc., were not very apparent.

The liver of *Heteropneustes fossilis* after exposure to 400ml of 1% sodium arsenite+zeolite solution for 3days showed disaggregation of hepatocytes as major histological change. At some points necrosis and inflammation were also visible but ballooning was not found in this section. After 7days exposure, section showed only mild disaggregation of cells. Even necrosis or inflammation was not very apparent and very little toxicological impact was noticed and on exposure to the same concentration for 15days, almost normal histopathological texture was seen. Any major histopathological alterations like ballooning, necrosis, inflammation and even disaggregation of hepatocytes were not found.

The results showing the effect of arsenic toxicity on histology of liver of *Heteropneustes fossilis* and its remediation using zeolite following exposure to different doses of sodium arsenite and sodium arsenite alongwith zeolite

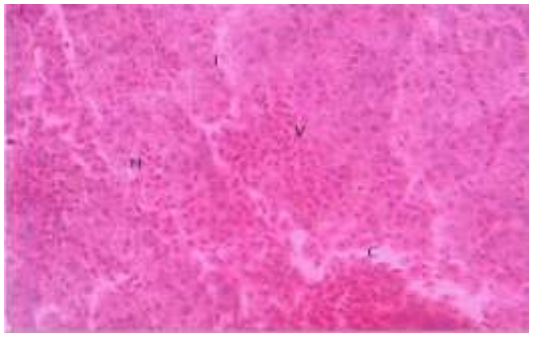


Plate 1: Photo-microphotograph showing liver of *Heteropneustes fossilis* (Control,100X).It shows lobules and hepatic acini.centered at portal tract T, and collagenous tissue (C),Hepatocytes(H) and hepatic venule(V).

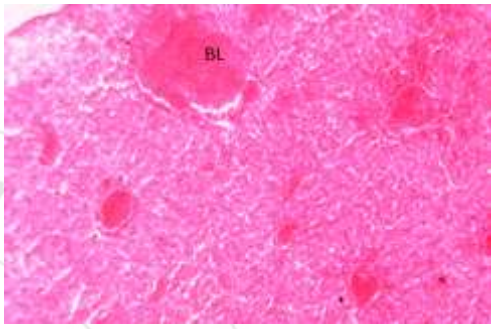


Plate 2: Photo micrograph of liver of *H.fossilis* after exposure to 200ml of 1% sodium arsenite solution for 3days.Single or scattered foci of cells have undergone swelling (BL-ballooning) due to accumulation of water in the cell probably due to proteins that are exposed.

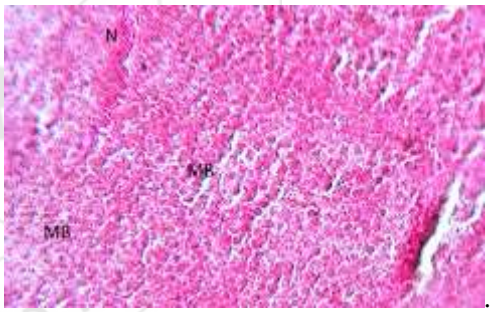


Plate 3: Photo-microphotograph showing liver of *H. fossilis* after exposure to 200ml of 1% sodium arsenite solution for 7days. It showed more inflammation in hepatocyte in comparison 3days exposure. Swelling (BL) and mallary body formation are very apparent, beside necrosis in cells.

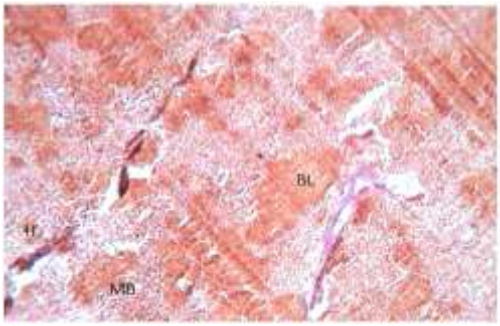


Plate 4: Photo-microphotograph showing liver of *H. fossilis* after exposure to 200ml of 1% sodium arsenite solution for 15days. A large number of swelled cells, hemosiderin and mallary bodies are very apparent

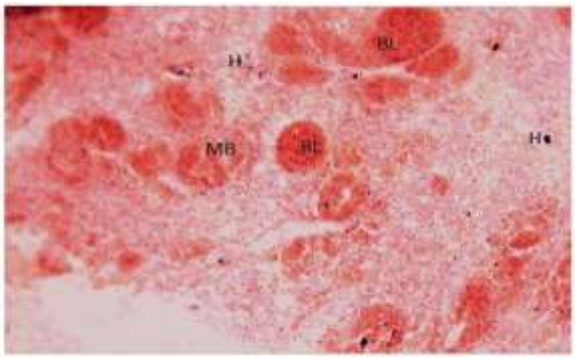


Plate 5: Photo-microphotograph of liver of *H. fossilis* after exposure to 400ml of 1% sodium arsenite solution for 3days, showing not only ballooning (BL) and necrosis in cell but also cholestasis. At several point hemosiderin (iron) (H) are also visible. At some point in section mallary bodies MB (i.e., scattered hepatocytes) are also developed.

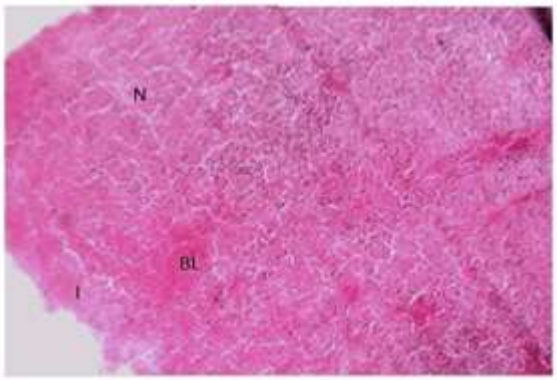


Plate 8: Photo-microphotograph of liver of *H. fossilis* after exposure to 200ml of 1% sodium arsenite+ zeolite solution for 3days, showing inflammation and ballooning at several point in hepatocytes. Necrosis and inflammation are also clear.

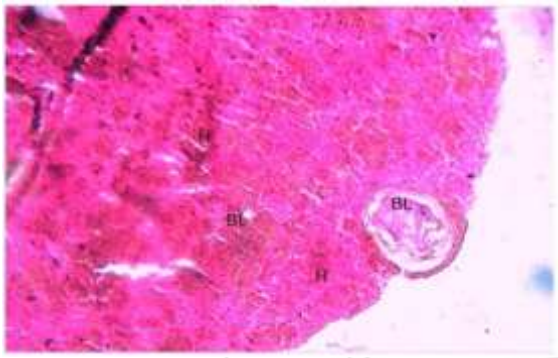


Plate 6: Photo-microphotograph of liver of *H. fossilis* after exposure to 400ml of 1% sodium arsenite solution for 7days, resulted in high level swelling in hepatocytees (BL) , as well ast hemosiderin (iron) (H) formation(H) and necrosis(N).

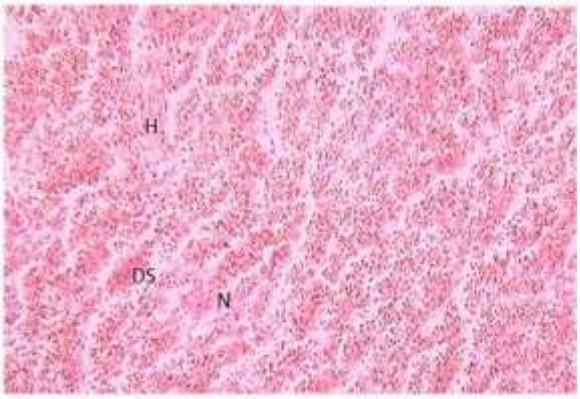


Plate 9: Photo-microphotograph of liver of *H. fossilis* after exposure to 200ml of 1% sodium arsenite+ zeolite solution for 7days, showing disaggregation of hepatocytes(DS) and necrosis(N) at some points.But ballooning was not found.

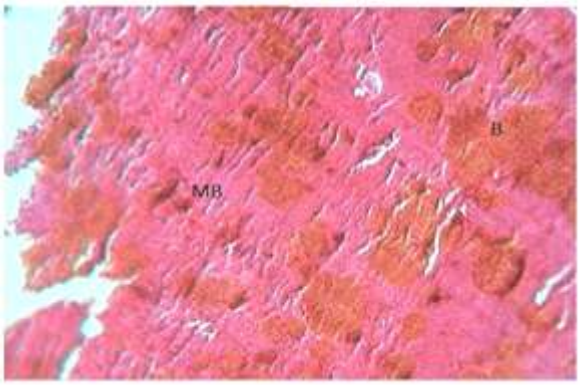


Plate 7: Photo-microphotograph of liver of *H. fossilis* after exposure to 400ml of 1% sodium arsenite solution for 15days, showing maximum histopathological damage. Large number of cells are showing necrosis and ballooning and mallary bodies..

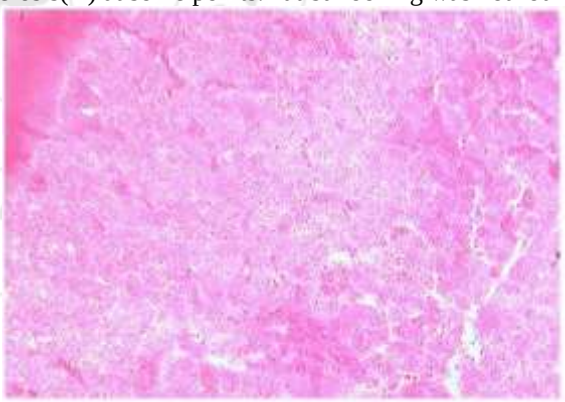


Plate 10: Photo-microphotograph of liver of *H. fossilis* after exposure to 200ml of 1% sodium arsenite+ zeolite solution for 15 days, showing almost normal histological texture.

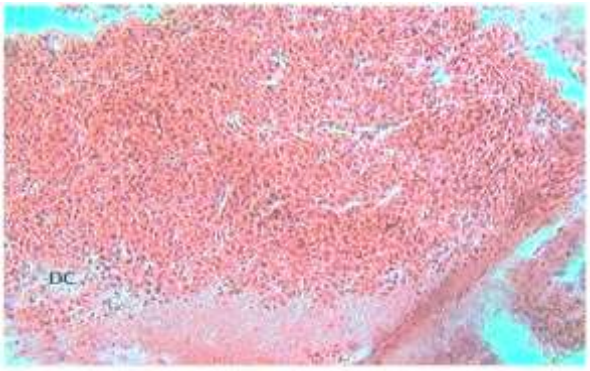


Plate 11: Photo-microphotograph of liver of *H. fossilis* after exposure to 400ml of 1% sodium arsenite+ zeolite solution for 7 days, showing only mild disaggregation hepatocytes

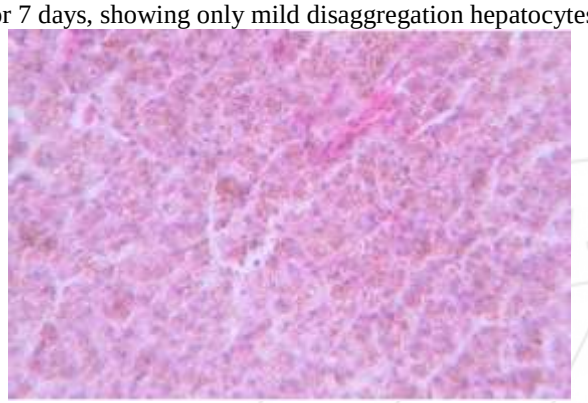


Plate 13: Photo-microphotograph of liver of *H. fossilis* after exposure to 400ml of 1% sodium arsenite+ zeolite solution for 15 days, showing almost normal histological texture

7. Discussion

Yusef *et al*. [34] reported altered biochemical parameters in rats exposed to sodium arsenite. A significantly decreased plasma total protein, albumin, and HDL cholesterol and increased urea creatinine, bilirubin, total lipid, cholesterol, triglyceride and LDL cholesterol were reported in arsenic treated rats

Effect of sodium arsenite on blood glucose, Hb concentration, PCV, MCV, MCH and MCHC of *Clarius batrachus* were studied by Tripathi *et al*. [28].A significant fall in hemoglobin concentration and PCV, MCV and MCH were reported.

Effect of acute and chronic exposure of sodium arsenite on total protein, albumin and globulin in serum of *Oryctolagus cuniculus* was reported by Tripathi *et al*. [29]. Animals were exposed to two sub-lethal concentrations of sodium arsenite for 3, 7and 15 days duration to determine acute toxicity and 6 months for chronic toxicity. The research reported decreased levels of total protein, albumin and globulin following acute and chronic exposures.

Result of a study by Palaniappan *et al*. [20] suggested that arsenic exposure could cause significant changes in bio chemical constituents like protein, lipids and nucleic acids, in gill tissues of *Labeo rohita*, and treatment with meso2, 3-dimercapto succinic acid (DMSA) could improve the levels of bio chemical constituents significantly.

Report from the studies of James and Sampath [14] stated that addition of zeolite to the cadmium contaminated media ($P<0.05$) reduced the cadmium level in water and fish body, and improved the biochemical parameters. It was reported that application of 4gm zeolite per litre to metal polluted water could reduce the cadmium toxicity in fish. Briggs and smith [4] also had suggested application of zeolite in ponds before stocking fries, to reduce heavy metal toxicity.

Decrease in soluble protein on exposure of *Heteropneustes fossilis* to Lead was reported by Jain [13]. It was also reported that the result obtained showed the ability of zeolite in decreasing the adverse effects. Martinez *et al.*, [17], had reported a significant reduction in blood protein in fishes (*Prochilodus lineathus*) exposed to Lead.

As the major toxins and drugs metabolizing and detoxifying organ in the body, the liver is subject to potential damage from an enormous array of pharmacological and environmental chemicals. The common morphological injury to liver cells caused by different toxins is hepatocyte swelling and necrosis, mallorybodies' formation, neurotropic reactions, fibrosis and cirrhosis.

Some authors have reported similar histopathological alterations in liver under arsenic intoxication. Ferzand *et al*., [8] have examined effect of arsenic toxicity in liver of mice and reported gross lesions of haemorrhages, necrosis, and degenerative changes in hepatocytes, hydroid and fatty degeneration of cells, cytoplasmic vacuole formation and blebbing.

Hemalatha *et al*., [10] reported degenerative change in liver cell of rats after arsenic intoxication. They have reported moderate fatty change under arsenic intoxication. In a case study Benramdane *et al*., [1] have also reported that after acute intoxication the liver and kidney have accumulated higher concentration of arsenic.

Santra *et al.*, [23] conducted a study on arsenicosis and their results are more or less similar to our finding and they also concluded that gross lesions and apoptosis of liver cells were due to oxidative stress in mitochondria, which played important role in the pathogenesis of arsenic induced apoptosis of the cells, as also evident by the study of Somia *et al*., [27] and Gupta *et al*., [9].

8. Conclusion

The results obtained showed that arsenic could alter protein metabolism in fishes and the use of zeolites was effective in protecting them against the toxic effects by reducing the adverse effects of sodium arsenite.

The most significant finding of present study is that zeolite treated arsenic did not cause any significant histopathological alterations in liver cells due to good chelating effect of zeolite for arsenic. The chelating effect of zeolite for arsenic toxicity was not properly examined in earlier works. 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 metal,

arsenic load may also be reduced from water and aquatic fauna, by using zeolite.

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