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IMPACT OF ARSENIC TRIOXIDE ON TOXICOPATHOLOGICAL PROFILE OF TELEOST, *CLARIAS BATRACHUS*

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ABSTRACT

The present investigation deals with the effect of arsenic trioxide on *Clarias batrachus* after 30, 45 and 60 days of exposure period. First group was treated as control group. Fishes of other 3 groups were treated with sublethal concentration 8.7mg/l of Arsenic trioxide for period of 30, 45 and 60 days. After exposure to experimental chemical, arsenic trioxide the liver cells of *Clarias batrachus* after 30 days showed Vacuolization, necrosis, cell damage, nuclear hypertrophy, cellular hypertrophy. Hypertrophy and cellular hypertrophy, cytoplasmic vacuolization was seen after 45 days of arsenic exposure while 60 days of exposure period showed necrotic cells, karyolysis and Kayorrhexis. edema between sinusoidal cells.

KEYWORDS: Karyolysis, Kayorrhexis, Oedema.

INTRODUCTION

Intense activities in industrial and agricultural sectors have inevitably increased the levels of heavy metals in natural waters. Heavy metals play a major role among pollutants of environmental concern.[1] They are serious pollutants of aquatic environment because of their environmental persistence and ability to be accumulated by aquatic organisms.[2] The name “Arsenic” is derived from the Greek word “arsenikon”, which means yellow orpiment. It ranks 20th in earth's crust, 14th in sea water and 12th in human body, exhibiting metallic as well as non-metallic characteristics and corresponding chemical properties. Hence, it is known as metalloid, a well-known 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 consequence of pollution. Arsenic is one of the oldest human poisons

known to mankind. It has six specific characteristics.[3] It has been considered as virulent poison on acute ingestion and is extremely toxic on long term exposure to very low concentrations, not visible in water and food, having no taste and no smell. Arsenic is a difficult metal for analysis, even when occurring in concentration twice as high as WHO guidelines, found in various chemical forms and oxidation states and is released into the aquatic environment by various process and industrial discharges. After releasing to aquatic environment, arsenic species enter into methylation / demethylation cycle, while some are bound to the sediments or taken up by biota where, they could undergo metabolic conversion to other organo-arsenicals. 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 AsO4 $_3$-, and H2AsO_4-. 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. The changes in the haematological parameters of fish are a helpful biomarker for evaluating their health status.[4] Pure arsenic is usually found in the environment combined with other elements such as Oxygen, Chlorine called as inorganic arsenic. Arsenic combined with carbon and hydrogen is called arsenic (organic). Two primary forms of Arsenic in water are trivalent arsenic (As+3) arsenite(III) and pentavalent arsenic (As+5) arsenate (V). Arsenic as free element (o-oxidation) state is rarely encountered in natural waters. Soluble inorganic arsenate predominates under normal conditions since it is thermodynamically more stable in water than arsenite (+3) oxidation state. The most important inorganic arsenic compounds are arsenic trioxide, sodium arsenite, arsenic trichloride, arsenic acid and arsenites (trivalent forms) lead and calcium arsenates (pentavalent forms). Inorganic arsenic is more toxic than organic and the trivalent forms are more toxic than pentavalents.[5] Common organic arsenic compounds are arsanilic acid, methylarsonic acid (MMA), dimethylarsinic acid (DMA), and arsenobetaine.[6] In the nature, arsenic can also be found to a small extent in elemental form. Arsenic is one of the toxic environmental pollutants which has recently attracted attention because of its chronic and epidemic effects on human health. The biotransformation of inorganic arsenic species (As+3 and As+5) into less toxic organic species can occur through monomethylarsonic acid (MMAV) reductase and methyltransferase enzymes involvement during reduction and methylation reactions.[7][8] General inorganic arsenic species like As +3 and As +5[9] are more toxic than organic species, in spite of the differences that exist between the effects of arsenite

(As+3) and arsenate (As+5).[10][11][12] Among the inorganic arsenic species arsenate is less toxic when compared to arsenite both under in vivo and in vitro conditions. The toxicity of arsenite (As+3) is related to its high affinity to sulphydryl (–SH) groups of proteins like glutathione (GSH) and lipoic acid and the cysteinyl residues of many enzymes while arsenate interferes with phosphorylation reactions.[13] 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 aggrevated 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[14], an important environmental contaminant is present in the aquatic environment as a result of geogenic and anthropogenic processes,[15][16] Liver is a major target organ of arsenic toxicity. As the principal metabolic organ, fish liver plays a major role in uptake, accumulation, bio-transformation and excretion of arsenic,[17] It has been reported that sublethal concentration of arsenic induces stress proteins[18] interefers with expression of different stress related genes[19] and helps in generation of oxidative stress in fish liver.[20] In aquatic environment, fishes are usually regarded as organisms of choice for assessing the effects of environmental pollution on aquatic ecosystems.[21]

MATERIALS AND METHODS

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. They were brought to the laboratory as soon as to lessen the high mortality. Prior to the experimentation, fishes were thoroughly washed for 5 minutes with 0·01% Kmno4 to avoid any dermal infection. Selected fishes were acclimatised to the laboratory conditions for period of 15 days.

ANIMAL CARE AND MAINTENANCE

The fishes were maintained in glass aquarium under natural photo period. The water quality, dissolved oxygen content, pH were monitored regularly in each aquarium. Diseased fishes showing any abnormal behaviour were removed immediately from the tanks. Fishes were fed ad libitium with minced chicken liver with commercially available fish feed. Fishes were fed twice a day. Water in the aquariums were renewed after every 24 hr to get rid of faecal matter.

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 sublethal concentration 8.7mg/l of 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.

PREPARATION OF STOCK SOLUTION AND DETERMINATION OF 96 HOUR LC50 VALUE OF ARSENIC TRIOXIDE: 1gm of Arsenic trioxide stock solution was prepared by dissolving arsenic trioxide in 1NHCl 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. Nine replicates of fish were maintained for each concentration and 20 fishes of equal size and weight were introduced. The test water was renewed at the end of 24 h and freshly prepared Arsenic trioxide was added to maintain the concentration of arsenic at a constant level. A concurrent control of 20 fish in three different glass aquariums were maintained under identical conditions. The mortality/survival of fish was recorded after 96 h. The dead fishes were removed from the tank immediately. The concentration at which 50% mortality of fish occurred after 96 h was taken as the median lethal concentration (LC 50), which was 87 mg/l, 1/10th value of the LC 50 value for 96 h (8.7 mg/l) was taken as the sublethal concentration.

HISTOPATHOLOGICAL STUDIES: After termination of experimentation period, the fishes were killed by damaging the brain liver were immediately removed from control and treated fishes for histopathological studies. After washing, dehydration in graded alcoholic series and clearing in xylene, the tissues were embedded in paraffin wax at 60^0C. Addition of little histopatho-wax was found useful in lowering the melting point and in getting a continuous ribbon. Section of 5 mm were cut and stained with haematoxylin/eosin for the light microscopic examinations. Microphotographs of the sections were taken. Most of microphotograph has been taken in low as well as high magnification in order to study various histopathological changes.

OBSERVATION

The liver of normal fish, *Clarias batrachus* consist of polygonal hepatocytes. Each hepatic cell consists of distinct nucleus, central nucleolus with granular cytoplasm. Hepatocytes form a rather cord-like pattern, these cords are arranged around tributaries of the hepatic vein. **(Fig 1)** A large number of blood sinusoids were observed which separates the hepatic cords one from another. Bile caniculi between the two layers of cells form network of ducts eventually draining into canal of herring which entered in portal canal and merged into fine branches of bile duct. **(Fig 2)** After exposure to experimental chemical, arsenic trioxide the liver cells of *Clarias batrachus* were found to loose their regular shape due to precipitation of both cytoplasmic and nuclear material which resulted in shape deformation. Vacuolization, necrosis, cell damage, nuclear hypertrophy, cellular hypertrophy were the prominent alterations observed at this stage. This stage was well documented with arsenic-induced changes characterized by dilation of sinusoids, formation of intracellular edema, megalocytosis, and vacuolation. Hepatic cells with distorted nuclei were observed 45 days of exposure period were marked by degenerative changes in nucleus and cytoplasm which was noted in the form of nuclear hypertrophy and cellular hypertrophy, cytoplasmic vacuolization was observed during this period. Pyknosis, as well as necrosis was also noted at this stage. **(Fig 3)** After 60 days of treatment severe degenerative changes were observed around the central vein, which were marked by dialation of central vein showing rupture at various places. Nuclear changes were observed around the central vein, which showed pyknosis as well as karyolysis. **(Fig4):** Degenerative changes were observed all around the portal tract and central vein. Hepatocytes showed necrotic cells, karyolysis and Kayorrhexis. edema between sinusoidal cells was more pronounced.

DISCUSSION

Arsenic trioxide exposed fish exhibited considerable changes in liver sections and in the cellular components. The first sign of arsenic trioxide induced change is evident in the tissue sections obtained from 30 days exposed fish, with swollen hepatocytes and the sinusoids between hepatocellular plates becoming slightly dilated with initiation of vacuolation and glycogen deposition was also noted. The highest degree of histological changes were observed in the sections from 30-day arsenic- trioxide exposed liver in which the hepatocytes were enlarged (megalocytosis) due to an increase in nuclear–cytoplasmic ratio and appeared rather disorganized. It was further noted that the sinusoids had became more dilated leading to intracellular edema. Cells with distorted nucleus (necrotic) were also observed in the 45

days exposed fish liver. Liver sections from 30-day exposed fish exhibited architectural loss in the hepatocyte appearance of necrotic cells. Several studies are reporting arsenic-induced liver fibrosis, hepatocellular damage, inflammation, focal necrosis in addition to hepatocellular carcinoma these observations coincides with studies of various workers.[22][23][24][25] After 30 days of exposure the normal architecture of the liver was altered. The hepatocytes show hydrophic degeneration and degenerative changes in nucleus. The central vein was dilated, blood sinusoids show dilation and congestion. Liver enlargement was observed in the present investigation which may be due to arsenic induced hepatocyte formation of vacuoles, oedema observed in the liver of exposed fishes are similar to those observed by workers in their studies.[26][27][28] After 45 days of exposure the hepotocytes show cloudy swelling, Nuclear hypertrophy, cellular hypertrophy with cytoplasmic vacuolization, nuclear atrophy, peripheral nuclei, cytoplasmic degeneration, cell rupture, nuclear degeneration these studies were similar to the studies of workers.[29][30][31] After 60 days of treatment severe degenerative changes were observed around the central vein, which was marked by dilation of central vein, showing rupture at various places. Nuclear changes were observed around the central vein, which showed pyknosis as well as karyolysis. Degenerative changes were observed all around the portal tract and central vein. Hepatocytes showed necrotic cells, karyolysis and Kayorrhexis. Oedema between sinusoidal cells were more pronounced. These studies were in aggrement to the earlier reports of workers[32[33][34][35] various places. Nuclear changes are observed around the central vein, which showed pyknosis as well as karyolysis. Degenerative changes were observed all around the portal tract and central vein. Hepatocytes showed necrotic cells, karyolysis and Kayorrehexis. Oedema between sinusoidal cells was more pronounced various places. Nuclear changes are observed around the central vein, which showed pyknosis as well as karyolysis. Degenerative changes were observed all around the portal tract and central vein. Hepatocytes showed necrotic cells, karyolysis and Kayorrehexis. Oedema between sinusoidal cells was more pronounced.

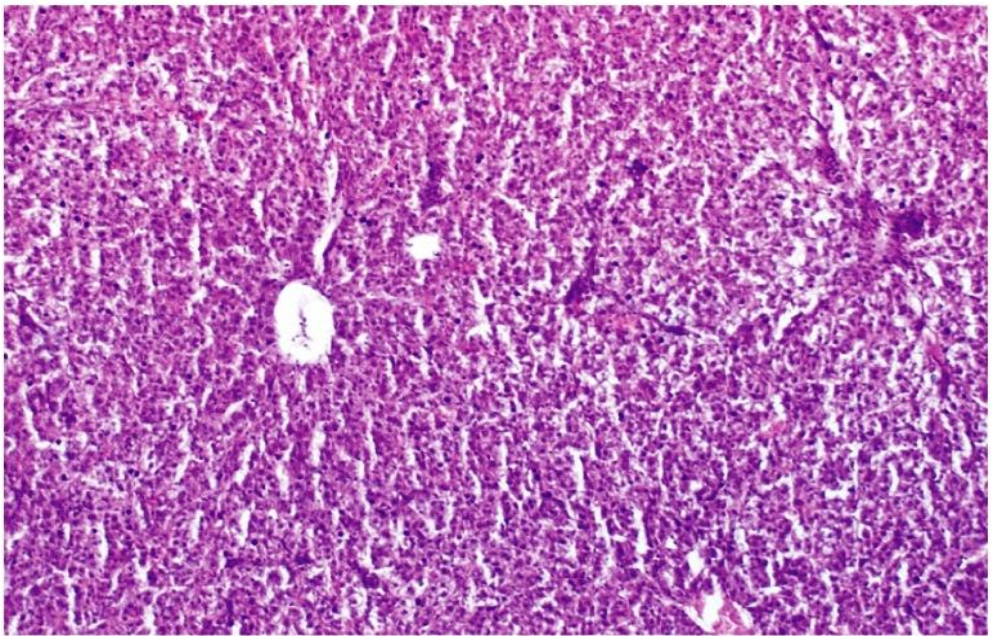

Fig 1: Microphotograph of T.S of Liver of control *Clarias batrachus* H.E X 200.

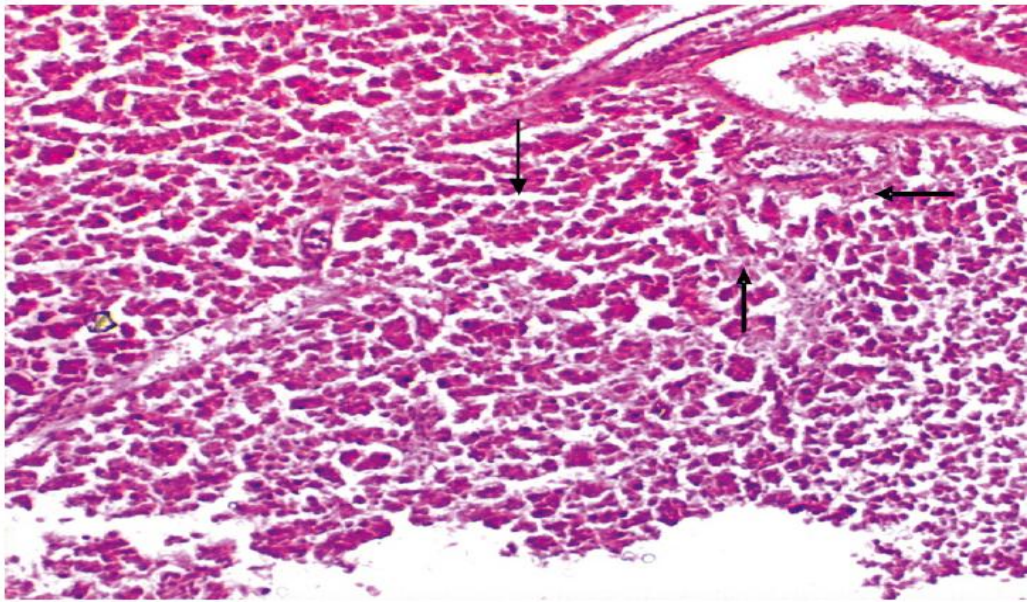

Fig 2: Microphotograph of T.S of Liver of *Clarias batrachus* after 30 days of exposure to arsenic trioxide exhibiting (megalocyte (→) intracellular oedema, (↑) Karyorrhexis, (←) cytoplasmic vacuolization, dilation of blood vessels H.E X200.

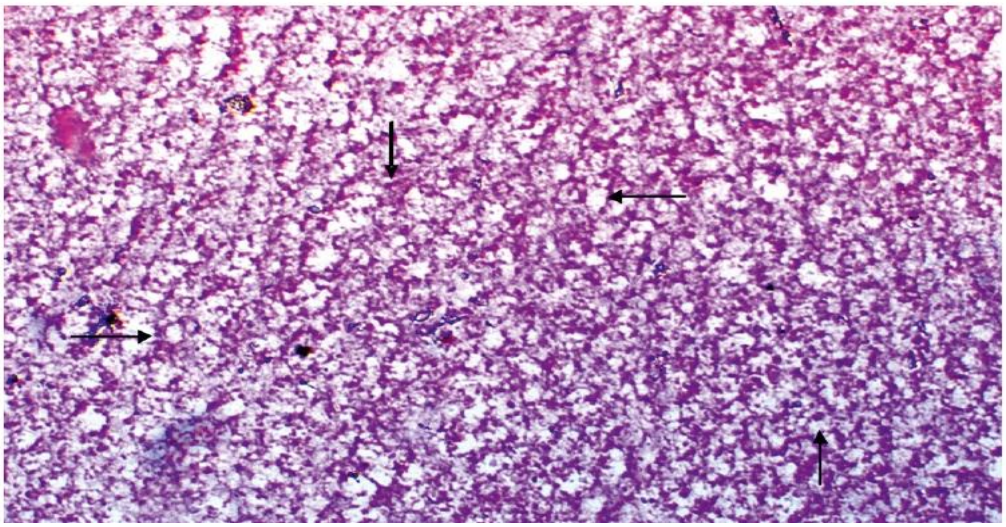

Fig 3: Microphotograph of T.S of Liver of *Clarias batrachus* after 45days of exposure to arsenic trioxide exhibiting (←) pyknosis (→)necrosis of hepatocytes, (↓) degenerative changes in nucleus, (↑) cellular hypertrophy, inflammation, coagulative necrosis H.E X200.

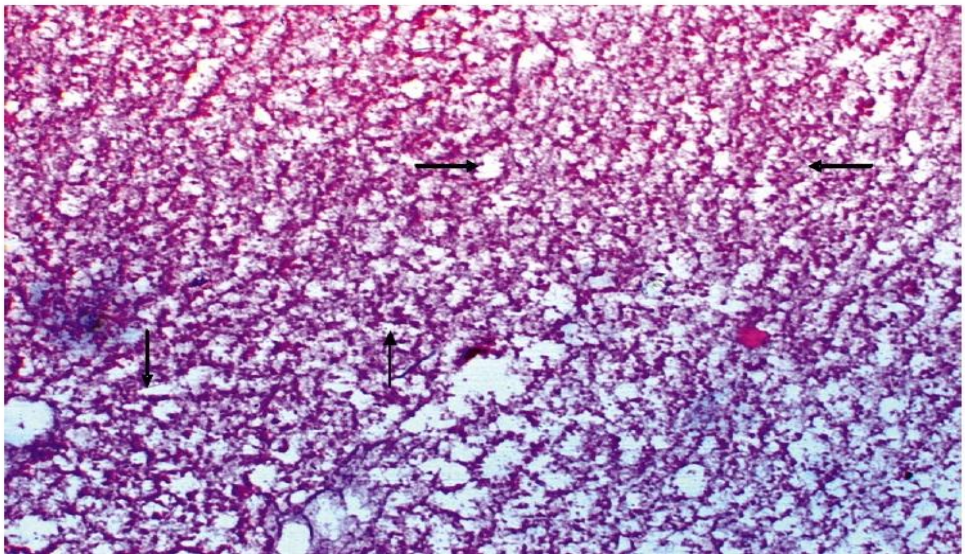

Fig 4: Microphotograph of T.S of Liver of *Clarias batrachus* after 60 days of exposure to arsenic trioxide exhibiting (←) pyknosis (↑) Karyolysis, (→) coagulative necrosis, (↓) hyperplasia and hypertrophy of hepatocytes, dilation of sinusoids, oedema H.E X 200.

CONCLUSION

30 days of exposure exhibits vacuolization, necrosis, cell damage, nuclear hypertrophy, cellular hypertrophy, histological studies on liver documented arsenic-induced changes characterized by dilation of sinusoids, formation of intracellular edema and megalocyte formation. Nuclear hypertrophy and cellular hypertrophy, cytoplasmic vacuolization was

observed during this period. Pyknosis, as well as necrosis was also noted after 45 days of exposure. 60 days of exposure period showed dialation of central vein, showing rupture at various places. Nuclear changes are observed around the central vein, which showed pyknosis as well as karyolysis. More degenerative changes were observed after 60 days of exposure period. Hepatocytes showed necrotic cells, karyolysis and Kayorrehexis. Oedema between sinusoidal cells was more pronounced.

REFERENCES

1. Singer C, Zimmermans. Induction of heat shock protsein in zebra mussel (*Dreissenapolymorpha*) following exposure to platinium, palladium and rhodium) comparision with lead and cadmium exposure *Toxicology*, 2005; 75: 654-675.
2. Veena KB, Radhakrishnan CK, Chacko J. Heavy metal induced biochemical effects in an estuarine teleost. *Indian J. Mar. Sci*, 1997; 26: 74-78.
3. Azcue J M and Nriagu JO. Arsenic Historical Perspective. In : *Arsenic in Environment.*, 1994; Part I. John Wile and Sons; London.
4. Rehulka J. Influence of astaxanthin on growth rate, condition and some blood indices of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, 2000; 190: 27-47.
5. Akter KF, Owens G, Davey DE, Naidu R.Arsenic speciation and toxicity in biological systems. *Rev. Environ. Contam. Toxicol*, 2005; 184: 97–149.
6. FribergL, Nordberg GF, and Vook VB. Handbook on the toxicology of metals, 2nd edition, *Elsevier Science Publisher*, Amsterdam, New York, 1986; II: 43-83.
7. Vahter M. - Mechanisms of arsenic biotransformation. *Toxicology*, 2002; 181-182: 211-217.
8. Akter KF, Owens G, Davey DE, Naidu R. Arsenic speciation and toxicity in biological systems. *Rev. Environ. Contam. Toxicol*, 2005; 184: 97-149.
9. Tseng C. The potential biological mechanisms of arsenic- induced diabetes mellitus. *Toxicol. Appl. Pharmaco*, 2004; 197: 67-83.
10. Tseng C. The potential biological mechanisms of arsenic- induced diabetes mellitus. *Toxicol. Appl. Pharmac*, 2004; 197: 67-83.
11. Akter KF, Owens G, Davey DE, Naidu R. Arsenic speciation and toxicity in biological systems. *Rev. Environ. Contam. Toxicol*, 2005; 184: 97–149.
12. Tuzen M, Cıtak D, Mendil D, Soylak M. Arsenic speciation in natural water samples by coprecipitation-hydride generation atomic absorption spectrometry combination. Talanta, 2009; 78(1): 52–56.

13. Andrew AS, Warren AJ, Brachowsky A, Temple KA, Klei L, Soucy NV, Oara KA, Hamilton JW. Genomic and proteomic profiling of responses to toxic metals in human lung cells. *Environ. Health Perspect,* 2003; 111: 825–835.
14. Tamaki S, Frankenbeger WT. Environmental biochemistry of arsenic. *Rev Environ Contam Toxicol,* 1992; 124: 79-110.
15. Gonzalez HO, Roling JA, Baldwin WS, Bain LJ. Physiological changes and differential gene expression in mummichogs (*Fundulus heteroclitus*) exposed to arsenic. *Aquat. Toxicol.*, 2006; 77: 43–52.
16. Singh AK, Banerjee TK. Toxic effect of sodium arsenate ($Na_2HAsO_4.7H_2O$) on skin epidermis of air breathing catfish *Clarias batrachus* (L.). *Veterinarski Arhiv*, 2008; 78(1): 73-88.
17. Pedlar RM, Klaverkamp J F. Accumulation and distribution of dietary arsenic in lake white fish (*Coregonus clupeaformis*). *Aquat. Toxicol,* 2002; 57: 153–166.
18. Roy S, Chattoraj A, Bhattacharya S. Arsenic-induced changes in optictectal histoarchitecture and acetylcholinesterase–acetylcholine profile in *Channa punctatus:* amelioration by selenium. *Comp. Biochem. Physiol. C, Comp. Pharmacol. Toxicol,* 2006; 144: 16–24.
19. Bears H, Richards JG, Schulte PM. Arsenic exposure alters hepatic arsenic species composition and stress mediated-gene expression in the common Killifish (*Fundulus heteroclitus*). *Aquat. Toxicol*, 2006; 77: 257–266.
20. Bhattacharya AK, Mandal SN and Das SK. Bioaccumulation of chromium and cadmium in commercial edible fishes of gangetic West Bengal. Trends. *Applied. Sci. Res*, 2006; 1: 511-17.
21. Gernhöfer M, Pawert M, Schramm M, Müller E, Triebskorn R.Ultra structural biomarkers as tools to characterize the health status of fish in contaminated streams. *J. Aquat. Ecosyst. Stress Recovery*, 2001; 8: 241–260.
22. Liu SX, Athar M, Lipai I, Waldren C and Hei TK. Introduction of oxyradicals by arsenic, implication for mechanism of genotoxicity. *Proc. Nat. Acad. Sci.* USA, 2001; 98: 1643-1648.
23. ATSDR. Toxicological Profile for Arsenic. Agency for toxic Substances and Disease Registry. SUDHHS, PHS, Washington, 2002. DC.
24. Datta S, Saha D R, Ghosh D, Majumdar T, Bhattacharya S, Mazumder S.Sub-lethal concentration of arsenic interferes with the proliferation of hepatocytes and induces in

vivo apoptosis in *Clarias batrachus L.* Comp. *Biochem. Physiol. C. Comp. Pharmacol. Toxicol*, 2007; 145: 339–349.

25. Kotsanis N. and Georgudaki JL. Arsenic induced liver hyperplasia and kidney fibrosis in rainbow trout (*Oncorhynchus mykiss*) by microinjection technique: A sensitive animal bioassay for environmental metal toxicity. *Bull. Environ. Contam. Toxicol*, 1999; 62: 169-178.
26. Palackova J, Fasaick P T and Celechovska O.Sublethal effects of Cadmium on Carp (*Cyprinus carpio*) fingerlings. In: R. Müller and Lloyd, R (eds), Sublethal and chronic effects of pollutants on freshwater fish. Fishing New Books, London, 1994; 54-61.
27. Marty G D, Okihiro M S, Brown E D, Hanes D and Hinton D E. Histopathology of adult Pacific herring in Prince William Sound, Alaska after the Exon Valdez oil spill. *Can. J. Fish Aquat. Sci*, 1999; 56: 419-426.
28. NagyP, Teramoto, T., Factor, V., Sanchez, A., Sanchez, A., Schnur, J., Paku, S., Thorgeirrson, S.Reconstitution of liver mass via cellular hypertrophy in rat, *Hepatology*, 2001; 33: 339-345.
29. Yang J, Chen H. Serum metabolic enzyme activities and hepatocyte ultrastructure of common carp after gallium exposure. *Zool. Studies*, 2003; 42: 455-461.
30. Roy S, Chattoraj A, Bhattacharya S. Arsenic-induced changes in optic tectal histoarchitecture and acetylcholinesterase–acetylcholine profile in *Channapunctatus:* amelioration by selenium. *Comp. Biochem. Physiol. C, Comp. Pharmacol. Toxicol*, 2006; 144: 16–24.
31. Datta S, Ghosh D, Saha D R, Bhattacharaya S, Mazumder S. Chronic exposure to low concentration of arsenic is immunotoxic to fish: role of head kidney macrophages as biomarkers of arsenic toxicity to *Clarias batrachus. Aquat. Toxicol*, 2009; 92: 86–94.
32. Crunkhorn SE, Plant KE, Gibson GG, Kramer K, Lyon J, Lord PG, Plant NJ. Gene expression changes in rat liver following exposure to liver growth agents: role of Kupffer cells in xenobiotic-mediated liver growth. *Biochem. Pharmacol*, 2004; 67: 107–118.
33. Chen CM, Yu SC, Liu, M.C. Use of Japanese medaka (*Oryziaslatipes*) and tilapia (*Oreochromismossambicus*) in toxicity tests on different industrial effluents in Taiwan. *Arch Environ Contam Toxicol*, 2001; 40: 363–370.
34. Seok S-H, Baek M-H, Lee H-Y, kim D-J, Na, Y-R Noh K-J, Park S-H lee H-K, Lee B-H., Ryu, D-Y, Park J-H,. Arsenite – induced apoptosis is prevented by antioxidants in Zebra fish liver lines *Toxicol in vitro*, 2007; 2: 970-877.

35. Chen H, Li SF, Liu J, Diwan BA, Barrett JC, Waalkes MP. Chronic inorganic arsenic exposure induces hepatic global and individual gene hypomethylation: implications for arsenic hepatocarcinogenesis Carcinogenesis, 2004; 25: 1779–1786.