Biospectra : Vol.8(1), March, 2013, pp 57-66
An International Biannual Refereed Journal of Life Sciences

Animal Sciences

ISSN : 0973-7057

Arsenic induced histopathological and biochemical alterations in the kidney of fresh water fish *Clarias batrachus*

G.B. Chand[a]* & Pawan Kumar[a]
*[a]P.G. Department of Zoology, Patna University, Patna

Received , 05th January, 2013 ;Revised:10th February, 2013

Abstract : The present research work is aimed to investigate the deleterious impact of arsenic trioxide (AS_2O_3) on fresh water fish *Clarias batrachus* based upon histopathological studies of renal tissue by light microscopy as well as estimation of LPO (Lipid peroxidation). Healthy fish were collected from NMCH fish farm during pre-spawning season and acclimatized in the ideal laboratory condition for 15 days. Fish were fed with commercial pelleted diet @3-4% of body weight. The LC_{50} of commercial brand Arsenic trioxide (CAS No. A13629) was calculated as per standard APHA method and accordingly 1/ 6th, 1/4th and ½th of LC_{50} value were administered to the fish for one, two and four week respectively. After termination of each exposure blood samples were collected in a heparinized glass culture tube and serum was extracted and estimated for LPO by standard method. The renal tissues were fixed in aqueous Bouin's fixative and processed for light microscopy. 5µ thick sections were cut on rotary microtome and dehydrated through graded series of alcohol, double stained in haematoxylin and eosin, cleaned in xylene and mounted in DPX. The LM photography was done by Canon Ixus 130S digital camera. The data obtained were subjected to statistical analysis using paired 't' test and one way ANOVA test and considered significant at $P<0.05$. Arsenic showed a significant increasing trend in a serum LPO in different groups of treated fish over control. The major histopathological anomalies incurred in kidney were shrinkage and constriction in glomerular tuft, inflammation of podocytes, widening of urinary space, increased incidence of necrotic renal tubule, massive fibrosis of arcuate artery and arcuate vein, infilteration of lymphocytes and plasma cells in peritubular space. Coincidence of biochemical and histopathological findings clearly suggests that arsenic even at its sublethal dose affects the overall health status of fish.

Key words : ***Clarias batrachus,*** **Renal tissue, Light microscopy, Histopathology, LPO, Arsenic trioxide.**

INTRODUCTION

The environmental conditions have shown massive deleterious changes by anthropogenic interventions. Heavy metal and pesticides contamination of the aquatic ecosystem has attracted the attention of researchers globally.[1] They may have devastating impact on the ecological balance of the recipient environment and a diversity of aquatic organism including fish.[2-4] Amongst heavy metal arsenic is the most noxious pollutant which has been used in insecticide, fungicide, rodenticide and wood preservative.

Ground water with elevated concentration of arsenic has been recognized as a problem of global concern. The middle Ganga plain covering about 89% geographical area of Bihar (»94000 km^2) holds potential aquifers. According to Central Ground Water Board & Public Health Engineering Department, Govt. of Bihar (2002), the arsenic contamination of surrounding water in the area has reached up to 0.178 mg/L. According to Saha[5] there are 15 hotspots of arsenic contamination in Bihar. The bioaccumulation

*Corresponding author :
Phone: 0
E-mail :gbchand@rediffmail.com

of arsenic in various tissue and blood of fish and related abnormalities have been well documented.[6-14] Vinodhini & Narayanan[15] have reported various heavy metal induced histopathological alterations in selected organ of *Cyrinus carpio*. Since the kidney, being the major route of arsenic excretion, is seriously affected by arsenic poisoning. Various site of arsenic damages in the kidney include capillaries, tubule and glomerulus[16,17].

Lipid peroxidation (LPO) refers to the oxidative degeneration of lipid. Peroxidation of lipid can disturb the assembly of membrane, causing changes in fluidity & permeability, alteration of ion transport and ultimately inhibition of metabolic process. Heavy metals are known to increase LPO in fish.[18,19]

Arsenic contamination of ground water as well as that of shallow water in wetland of Gangetic plain has increased tremendously in last few decades and it is posing serious threat to survival of fishes of worth importance. Hence the present investigation is designed to elucidate a systematic deleterious impact of arsenic on some of the oxidative parameters and histopathology of kidney of *Clarias batrachus.*

MATERIALS AND METHOD

Experimental animal – *Clarias batrachus* (Linn.) commonly known as "Mangur" was used as experimental model. It is hardy and restraining to most of the adverse ecological conditions as well as mild pesticide toxicity.

It has a fast growth rate and prolific breeding ability. It contributes 50%-60% of total Inland brood cat fish culture. It has been given the status of fish of state by Govt. of Bihar.[20] Different age groups of *Clarias batrachus* of mean body length 18±2 cm & mean body wt. 74±6 gm were collected from NMCH fish farm, Patna during pre-spawning season which is situated at 25°23' N and 85°21E on the southern bank of holy river Ganga. The fish were brought to the laboratory, disinfected with 0.1% $KMNO_4$ solution and acclimated in the ideal laboratory condition for 15 days with a constant supply of dechlorinated tap water at 27.7±0.24°C. Fish were fed *ad libitum* @ 2.5% of their body wt.

Chemical Used:

In experimental protocol, commercial brand arsenic trioxide (AS_2O_3) C.No.A13629, manufactured by Nice Chemicals Pvt. Ltd., Cochin was used. The LC_{50} of AS_2O_3 for fish was calculated by standard APHA (2005)[21] method as 150 mg/l (As as 114 mg/l). 1/6th, 1/3th and 1/2th of LC_{50} value of AS_2O_3 (25mg/L, 50 mg/L and 75 mg/L) were considered for one, two and four week respectively. Accordingly stock solutions were prepared.

Blood Sample Collection:

After the termination of each exposure the fish were rinsed with deionized water and then anesthetized in pH neutralized tricaine methane sulfonate-MS222 (Sigma). Blood samples were collected in a heparinized glass culture tube syringe by puncturing caudal vein and further drained into sterile eppendorf. The blood was centrifuged at 5000 rpm for 10 min. in Remi's high speed centrifuge. The supernatant containing serum was stored at -4°C for enzymatic assay of LPO. The LPO was estimated as per Ohkawa method[22] using spectrophotometer.

Histological preparation: For light microscopy, at the termination of each exposure, the different groups of anaesthetized fishes were sacrificed and kidney samples were fixed in aqueous Bouin's fixative. After 24 hours they were washed in running tap water. Tissues were dehydrated in graded series of alcohol, cleared in xylene and embedded in moulted paraffin (m.p.58°C) wax. Semithin sections of 5µ were cut on rotary microtome and processed for double staining using haemotoxylin and eosine and mounted in DPX. Approximately 2-4 sections of each group were analyzed on Olympus 2000 compound microscope. The photographs were taken using Canon ISUX 130S digital camera Microphotographs were printed and interpretations was done.

The acclimated fish were categorized into following groups –

01 – Group I Normal

02 – Group II 25 ppm arsenic trioxide (AS_2O_3) treated for one week

03 –Group III 25 ppm arsenic trioxide (AS_2O_3) treated for two week

04 – Group IV 25 ppm arsenic trioxide (AS_2O_3) treated for four week

05 – Group V 50 ppm arsenic trioxide (AS_2O_3) treated for one week

06 – Group VI 50 ppm arsenic trioxide (AS_2O_3) treated

for two week

07 – Group VII 50 ppm arsenic trioxide (AS_2O_3) treated for four week

08 – Group VIII 75 ppm arsenic trioxide (AS_2O_3) treated for one week

09 – Group IX 75ppm arsenic trioxide (AS_2O_3) treated for two week

10 – Group X 75 ppm arsenic trioxide (AS_2O_3) treated for four week

STATISTICAL ANALYSIS

For the estimation of serum LPO, six observations at random were taken. The arithmetic mean were calculated and subjected to paired 't' test and one way ANOVA test. The value at $P<0.05$ were considered significant. All the statistical analysis was done on sigma plot 8.0 version.

Table – 1 Showing LPO (m mole/ml) in different experimental Group of fish

Groups	Total No. of observations	LPO Level (μ mole/ml)		% increase (+) over control
		Mean	SEM	
Group I	6	1.435	0.832	-
Group II	6	1.920*	0.185	33.812 (+)
Group III	6	2.363*	0.540	64.661 (+)
Group IV	6	2.266*	0.528	57.895 (+)
Group V	6	2.784*	0.792	93.985 (+)
Group VI	6	3.151**	0.936	119.587 (+)
Group VII	6	6.820***	1.290	375.188 (+)
Group VIII	6	2.848**	0.047	98.496 (+)
Group IX	6	2.546*	0.006	77.443(+)
Group X	6	4.500**	0.228	213.534 (+)

Values are expressed in mean±SEM of six variants in each group. Paired t-test was applied between control (Group – I) and different arsenic treated group (II to X). Significant response:- * $P<0.05$, ** $P<0.01$, *** $P<0.001$. One way ANOVA test was done to observe overall variation in LPO. Calculated 'F' value 33.34 while table 'F' value is 2.18. Hence arsenic exposure significantly increases serum LPO in fish. Values in parenthesis are % increase (+) over control.

RESULTS AND DISCUSSION

Arsenic exposure of different doses in fish showed a characteristic declining trend which have been shown in text table –I

In the present study at lower concentration of As (III) serum LPO showed a significant ($P<0.05$) increase of 33.81% (Gr. II) and 64.66% (Gr.III) over control (Gr. I). Similar trend is maintained up to Group VI. A significant ($P<0.01$) increase of 375.18% over the control have been marked in Group VII fishes. A significant ($P<0.05$) increase of 98.49, 77.44 and 213.53% have been seen in Group VIII, IX & X fishes respectively. Similar kind of increasing trend in LPO content in the Indian catfish *Clarias batrachus* after exposure to low concentration of As (1-3 mM) have been reported[23]. However our findings are in contrast to those of Sounderarajan *et al.*[24], who claimed that arsenic exposure at low dose is not always associated with rise in LPO. Similar kind of increasing trend in LPO profile of liver and kidney and rats due to arsenic exposure have been elucidated.[25] Lipid peroxides (LP) are products of oxidative damaged lipids resulting from lipid peroxidation induced by reactive oxygen spaces.[26] Arsenic generated

free radicals have been reported to induce cell damage and cell death through the alteration of oxidative sensitive signaling pathway.[27] Generation of reactive oxygen species, alteration in the signal cascade and an imbalance in antioxidant level in turn triggers cellular apoptosis in cell.

A typical teleost kidney has two parts head kidney and trunk kidney. Trunk kidney contains renal corpuscles and tubules. The renal corpuscles are more spherical in shape containing highly vascular glomerulus (Plate – I, Fig.1, 2, & 3). The inner layer of Bowman's capsule is visceral layer consisting of podocytes, intermingled with endothelial cell while outer layer is known as parietal cell, made up simple squamous epithelial cell. The space between visceral and parietal layer is known as urinary space (Plate-I, Fig.1,2 & 3). Proximal convoluted tubule (PCT) is made up of large simple cuboidal epithelial cell having numerous brush borders. The inter digitations are more prominent in basal region (Plate-I, Fig,2 & 4). Distal convoluted tubules (DCT) are made up of large cuboidal epithelial cells. Boundaries between distal tubule cells are not distinct and it is partially devoid of brush borders (Plate – 1, Fig.1,2,3 & 4). The cells lining the collecting duct (CT) are made up of dark cells and light cells (Plate 1, Fig.4).

The transverse section of kidney of Group II fish showed massive deposition of edematous fluid in PCT, DCT and CT besides infiltration of plasma cell and polymorphonuclear cells, pus and fibrin in the peritubular space and lumen of DCT, marking a condition of polyelonephritis. (Plate II, Fig.2). In Group III fishes destructions were marked in epithelial cell lining of CT (Plate II, Fig.3). Calcification of kidney is well marked by a thick deposition of fibrous tissue on the inner elastic lamina of arcuate artery in Group IV fishes. In Group V fish kidney degeneration were marked in renal corpuscles and renal tubule as evidenced by constricted glomerular tuft, enlargement of Bowmann's space and presence of lymphocytes and edematous fluid in peritubular space (Plate III, Fig.1,2). A symptom of hydronephrotic kidney was marked in Group VI fish with increased number of necrotic renal tubule and massive infiltration of lymphocytes in interstitial space (Plate III, Fig.3). Kidney of Group VI fish showed acute glomerulonephritis and perivenular fibrosis with massive deposition of fibrous tissue in lumen of arcuate vein (Plate III, Fig.4).

At higher sub lethal exposure of arsenic, a typical sign of acute proliferative glomerulonephritis with a great reduction in glomerular capillary area & Browmann's space was marked. A massive infiltration of plasma cells in interstitial space and neutrophils & eosinophils in lumen of CT were marked in Group VIII fish (Plate IV, Fig.1,2). Group IX fish also showed shrinkage in the plasma cells filled lumen of DCT and CT due to inflamed endothelial cells (Plate IV, Fig.3). A distinct edematous fluid in the lumen of CT was seen in the transverse section of kidney of Group X fish (Plate IV, Fig.4).

Similar kind of dilation of lumen of kidney tubules, necrosis of tubules, shrinkage of glomerular tuft have been reported in *H. fossilis* exposed to chloropyrifos[28].

Elsan treatment in *Channa punctatus* resulted in a significant decrease in dimension of Bowmann's capsule & glomerulus and irregular shape of tubules due to precipitation of cytoplasm and karyolysis[29]. Similar dilation of renal tubules and various other necrotic changes in *L. rohita* exposed to hexachloro cyclohexane have been marked[30]. The circulatory disorders lead to recruitment of numerous macrophages and inflammatory cells, which develop necrosis around the border of tissues. It is probably the main cause for change in shape of kidney.[31]

The coincidence of biochemical and histopathological findings clearly reveals that arsenic exposure even at sub lethal level affects the overall health status of fish.

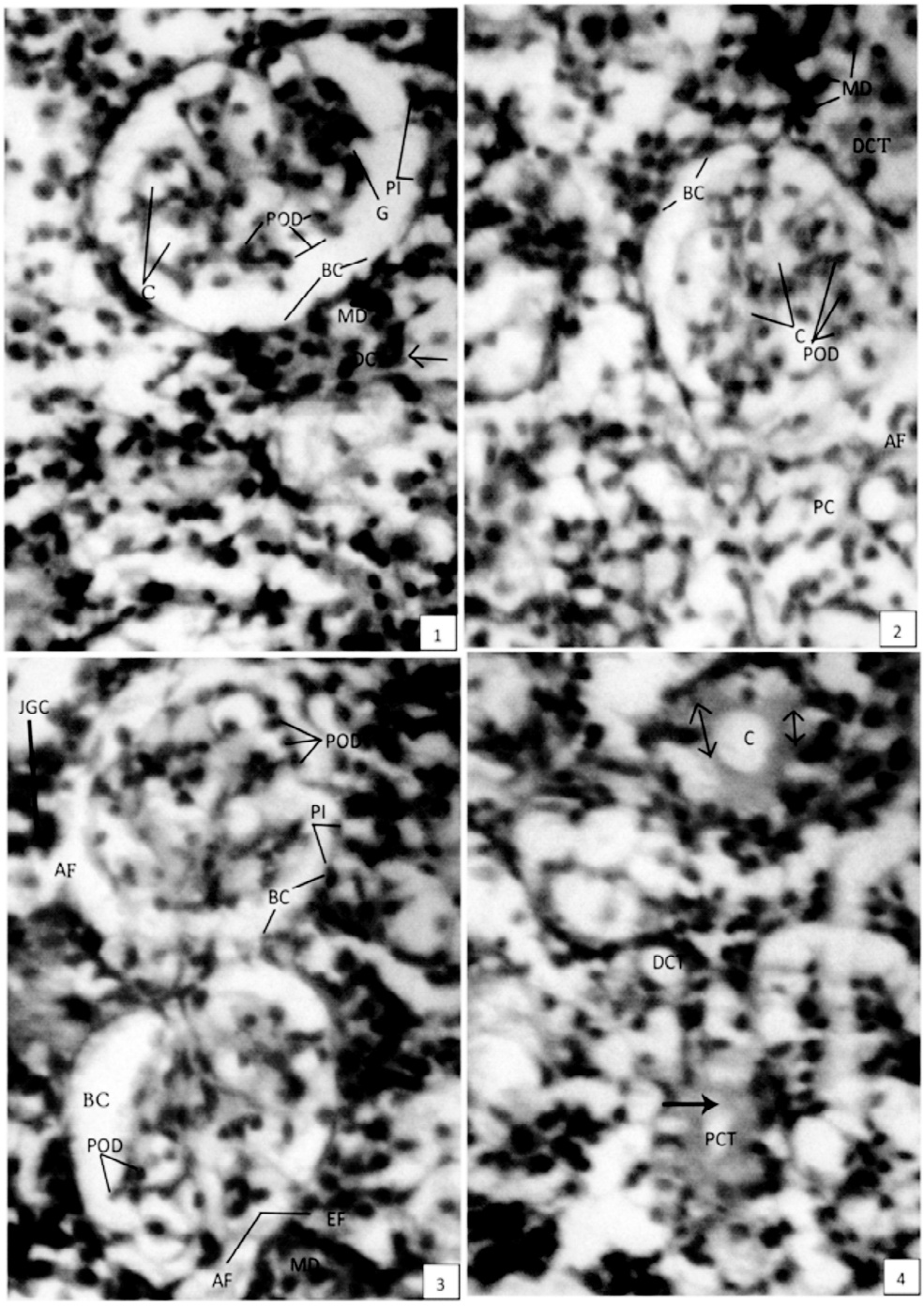

Plate – I: Photomicrographs of T.S. of normal kidney of *Clarias batrachus* showing detail normal histology.

Fig.1 & 2.showing renal corpuscles (RC) with distinct parietal layer lined by simple squamous epithelium & visceral layer lined by podocytges (Pod), macula densa (MD) of distal convoluted tubules (DCT) and few proximal convoluted tubules (PCT). (X100)

Fig.3. showing two adjacent renal corpuscles with afferent arteriole (AF) and efferent arteriole (EF). Fig.4. showing fire brush borders in lumen of PCT & prominent divisional marks in between neighboring cells in CT. (X400)

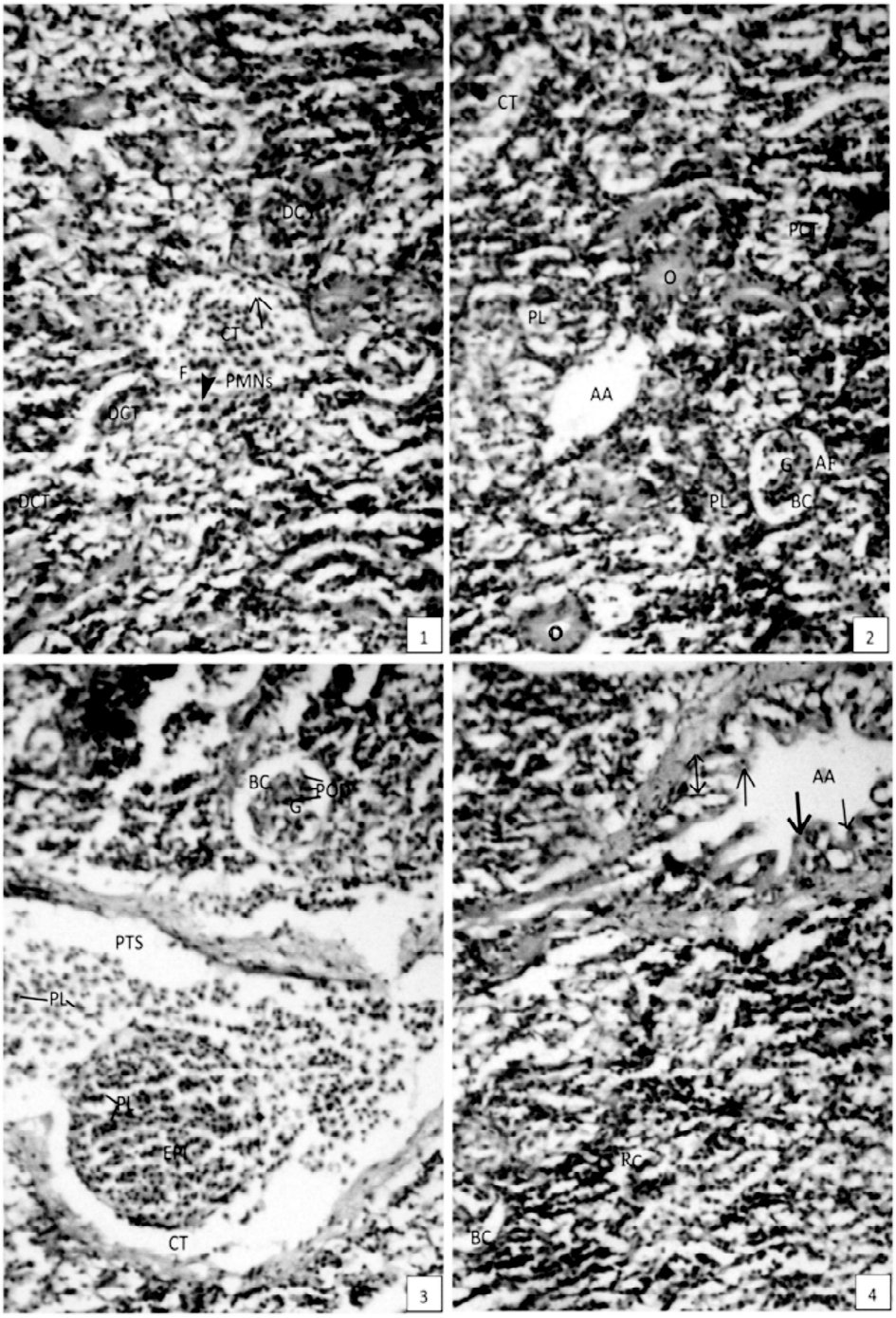

Plate – II : Photomicrographs of T.S. kidney of arsenic treated group (II-IV).

Fig.1. showing a condition of acute pyelonephritis, marked by the accumulation of pus & polymorphonuclear cells and fibrosis.

Fig.2. showing massive deposition of edematous fluid in DCT, PCT as well as CT.

Fig.3. showing distruction of epithelial cells lining collecting duct.

Fig.4. showing metastatic calcification of kidney. (X100)

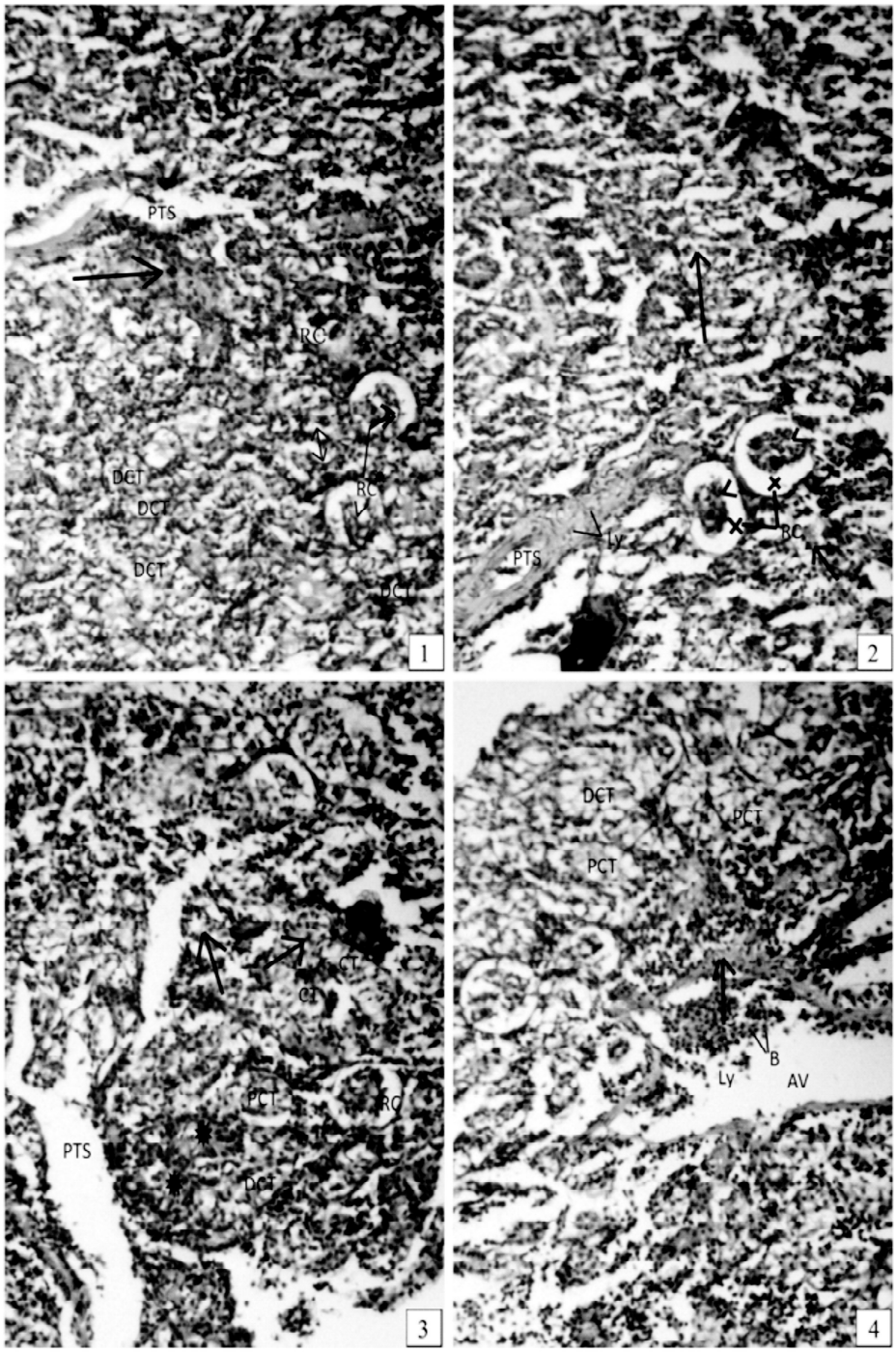

Plate – III: Photomicrographs of T.S. of kidney of arsenic treated Groups. (V-VII).

Fig.1 showing marked degeneration in renal corpuscles & renal tubules and prominent constriction of glomerular tuft.

Fig.2. showing enlarged Bowmann's space and increased no. of degenerative renal tubules surrounding renal corpuscles.

Fig.3. showing sign of hydronephrotic kidney with abundance of necrotic renal tubules and massive infilteration of lymphocytes in the interstitial space.

Fig.4 showing acute glomerulo nephritis with degenerating arcuate vein, renal corpuscles and renal tubules. (X100)

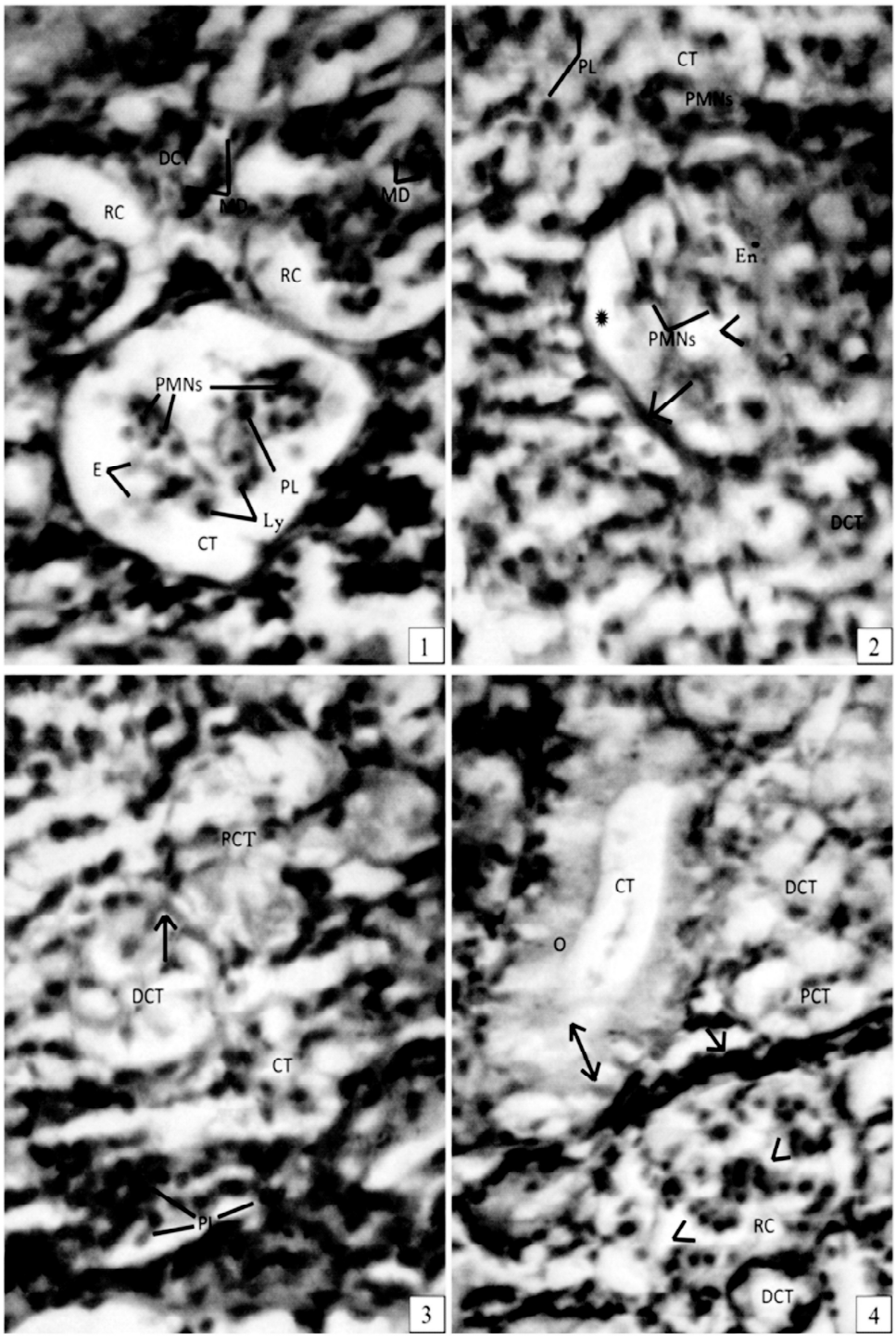

Plate – IV :Photomicrographs of T.S. of kidney of arsenic treated groups. (VIII-X).

Fig.1 & 2.showing typical sign of acute glomerulo nephritis with prominent reduction in glomerular capillary area and Bowmann's space, massive infiltration of plasma cell, neutrophils & eosinophils in interstitial space and lumen of CT. Fig.3 & 4. showing a condition of acute pyelitia with inflamed cells of CT and DCT and marked deposition of oedomatous fluid in their lumen. (X400)

ACKNOWLEDGEMENT

The authors are thankful to Head, Dept. of Zoology, Patna University, Patna for providing infrastructural facilities & UGC, New Delhi for providing fund through F.No.37-MRP-2009 (SR).

REFERENCES

1. **Dutta HM. & Dalal, R. 2008.** The effect of endosulfan on the ovary of blue gill sunfish: A histopathological study (*Lepomia machrochinus* sps.) *Int. J. Environ.* Res. 2 (3) : 215-224.
2. **Ashraf, W. 2005.** Accumulation of heavy metals in kidney and heart tissues of *Epinephelus microden* fish from the Arabian Gulf, *Environmental Monitoring and Assessment*. 101 (1-3): 311-316.
3. **Vosyliene MZ & Jan Kaite A.2006**. Effect of heavy metal model mixture on rainbow trout biological parameters, *Ekologija*, 4: 12-17.
4. **Farombi, E.O., Adelowo, O.A. & Ajimoko, Y.R. 2007**. Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African catfish (*Clarias batrachus)* from Nigera Ogun River, *Int. J. Environ. Res. Public Health*, 4(2):158-165.
5. **Saha, Dipankar. 2009.** Arsenic groundwater contamination in parts of middle Ganga Plain, Bihar. *Current Science*, Vol.97, No.6.: 25.
6. **Canli M. 1995.** Natural occurrence of metallothionein like proteins in the hepatopancreas of the Norway lobster *Nephrops norveigian* and effects of Cd, Cu and Zn exposure on levels of the metal bound on metallothionein. *Turk. J. Zool*., 19 : 313-321.
7. **Tort L & Tosses P. 1998.** The effects of sublethal concentration of cadmium on hematological parameters in the dog fish, *Scyliorhinus caniccula, J. Fish. Biol*., 32 (2): 277-282.
8. **Oliveira Ribeiro, Pelletier, C.E., Pfeiffer, W. & Rouleau C. 2000**. Comparative up take, bioaccumulation and gill damages of inorganic mercury in tropical and Nordic freshwater fish. *Environmental Research*, 83 (2000) 286-292.
9. **Filazi, A., Baskaya R, Kum, C. & Hismiogullari S.E. 2003.** Metal concentration in tissue of the Black sea fish *Mugil auratus* from Sinop-Icliman, Turkey, *Human and Experimental Toxicology*, 23 (2003) 85-87.
10. **Basa, S.P. & Rani, U.A.2003**. Cadmium induced antioxidant defence mechanism in freshwater teleost *Oreo chromis mossambicus* (Tilapia), *Eco. Toxicol. Environ. Saf*. 56(2) (2003) 218-221.
11. **Calta, M. and Canpolat, O. 2006.** The comparison of three cyprinid species in terms of heavy metals accumulation in some tissues. *Water Environmental Research*, 78: 548-551.
12. **Fernandez, C., Fontainhas-Fennandez, A., Peixoto, F. and Salgado, M.A. 2007.** Bioaccumulation of heavy costal lagoon, Portugal. *Ecotoxicology and Environmental Safety*, 66 : 426-431.
13. **Fernandez, C., Foutainhas-Fernandes, A., Cabral, D. and Salgado, M.A. 2008.** Heavy metal in water, sediment and tissues of *Liza sa*liena from Exmoriz-Paramos lagoon, Portugal, *Environmental Monitoring & Assessment*, 136 : 267-275.
14. **Mahomed Fatma, A.S. 2008**. Bioaccumulation of selected metals and histopathological alterations in tissues of *Oreochromis niloticus* and *Lates niloticus* from Lake Lasser, Egypt, *Global Veterinaria* 2(4) : 205-218.
15. **Vinodhini, R. and Narayanan, M. 2009.** Heavy metal induced histopathological alterations in selected organs of the *Cyprinus carpio* L. (Common carp), *Int. J. Environ. Res* 3(1) : 95-100.
16. **Schoolmester, W.L. and White, D.R. 1980.**, Arsenic poisoning, *South Med. J.* 73 : 198-208.
17. **Winship, K.A. 1984.** Toxicity of inorganic arsenic salts, *Adv. Drug. React Acute Poisoning Rev*. 3 (1984) 129-160.
18. **Rana, S.V.S., Singh, R. and Verma S. 1995.** Mercury-induced lipid peroxidation in the liver, kidney, brain and gills of a freshwater fish, *Channa punctatus*, *Japan. J. Ichthyol*. 42 (3/4): 255-259.
19. **Kumar, P., Prasad, Y., Ranjan, R., Swarup, D., Patnaik, A.K. & Patra, R.C. 2008.** Accumulation patterns of cadmium in tissues of Indian catfish *Clarias batrachus*, *Anim. Nutr. Feed Technol.* 8 : 115-119.
20. The status of fish of state by Govt. of Bihar, July, 2008.
21. APHA, Standard Methods for the Examination of water and waste water (21st Ed.). A joint publication of the American Public Health Association (APHA), the American Water Work Association (AWW) and the Water Environment Ferderarion (WEF), (2005) 1368 pages.
22. **Ohkawa, H., Ohisi, N. & Yagi, K. 1979.**, Assay of lipid peroxides in animal tissues by thiobarbituric reaction. *Annal. Biochem*. (1979) 95:351.

23. **Battacharya, A. and Battacharya, S. 2007.** Induction of oxidative stress by Ag in *Clarias batrachus* : Involvement of peroxisomes, *Ecotoxicol. Environ*. Saf. 66: 178-187.

24. **Soundarajan, M., Veeraiyan, G., Samipillai, S.S. 2009.**, Arsenic induced oxidative stress in freshwater catfish *Tilapia mossambia*. *Journal of Phytology Section: General Science*, 1 (4): 267-276.

25. **Sohini & Rana, S.V.S. 2007.** Protective effect of ascorbic acid against oxidative stress induced by inorganic arsenic in liver and kidney of rat. *Indian Journal of Exp. Biol.,* Vol. 95 (2007) 371-375.

26. **Yu, B.P. 1994.** Cellular defenses against damage from reactive oxygen species, *Physiol. Rev.* 74 (1994) 139-162.

27. **Shi, H., Shi, X. and Liu, K.J. 2004.** Oxidative mechanism of arsenic toxicity and carcinogenesis. *Mol. Cell Biochem.* 225 (2004) 67-78.

28. **Srivastava, S.K., Tiwari, P.R. and Srivastav, A.K. 1990.** Effects of chloropyrifos on the kidney of freshwater catfish *Heteropneustes fossilis*. *Bull. Environ. Contam. Toxicol*, 45 (1990) 748-751.

29. **Banerjee, S. and Bhattacharya, S. 1994.** Histopathoogy of kidney of *Channa puctatus* exposed to chronic nonlethal level of Elsan, mercury and ammonia. *Ecotoxicol. Environ. Saf.* 29 (1994) 276-75.

30. **Das, B.K. and Mukherjee, S.C. 2000.** A histopathoogical study of carp (*Labeo rohita*) exposed to hexachlorocyclohexane. *Veterinarski Arhiv.*, 70 (2000) 169-180.

31. **Vinodhini, R. and Narayanan, M. 2008.** Bioaccumulation of heavy metals in organs of fresh water fish *Cyrinus carpio* (Common carp), *Int. Environ. Sci. Tech*. 5(2) (2008) 179-182.

* * *