Chapter – VI

Histological, Histopathological and Histochemical Observation and Discussion on Kidney of Channa Striatus (Bloch.) Exposed to various Concentrations of Phosphate

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Kidney :

Review of literature :

The kidney is most important target organ which gets affected due to exposure to pollutants. The morphology, anatomy and histology of kidney is fishes have been extensively studied. Kidney is mainly associated with removal of harmful substances from the body. The histopathological alterations induced by pesticides, inorganic metals and phenolic compound and many other pollutants have reported to cause damage to fish kidney.

Amminikutty and Rege (1978) reported certain degenerative changes in the kidney of Nidow tetra (G. ternetzi) exposed to thiodan E.C. 35 and agallol '3' and their observations revealed that chronic exposure with thiodan resulted in an extensive increase of the haemopoietic tissue and with agallol edematous swelling of glomeruli and thickening of the wall of the Bowman's capsule. Endrin treatment resulted in shrinkage in glomerular network and desquamation of tubular cells (Shastry and Sharma, 1979). The sublethal concentration of sumithion showed changes in the kidney of C. batrachus showing vacuolation of epithelial cells of uriniferous tubules and degeneration of glomeruli (Mandal and Kulshrestha, 1980). Malathon toxicity was also studied in C. punctatus by Dubale and Shah (1984) and reported that maximum affected tissue

in the kidney was proximal tubules followed by injury to the glomerulus, haemopoietic tissue and proximal tubules. The effects of acute exposure to phosphamidon in kidney of N. denisonii showed shrinkage of glomeruli, cloudy swelling of the renal tubules, and biological effects indicated inhibition of alkaline phosphatase, G-6-phosphatase and LDH (Rashatwar and Ilyas, 1984). Similar observations were noted in kidney of A. testudineus exposed to furadan (Bhakthavathsalam et. al., 1984) of H. fossils (Gupta and Rajbanshi, 1979) in P. conchonium (Kumar and Pant, 1981) and in C. punctatus (Gupta and Rajbanshi, 1986) have been studied. Urea toxicity was extensively studied by Srivastava (1985). The effects of phenol and phenolic compounds showed histopathological changes in H. fossilis and in N. notopterus showing haemorrhage in glomeruli degeneration and dissolution of epithelial cells of renal tubules.

Histological observation on the kidney of control fish :

Histological architecture of Channa striatus comprised of numerous excretory units, the nephrons. Each nephron is formed of renal corpuscle, coiled uriniferous tubules and interstitial hematopoietic tissues. Renal corpuscle was made up of a glomerular and Bowman's capsule is formed (Plate No. 3, Fig. 1 and 2). Bowman's capsule is formed of inner squamoidal visceral epithelial cells and outer parietal layer of cuboidal cells. The neck is lined by a single layer of columnar epithelial cells. The proximal convoluted tubule was characterized by columnar cells with brush border along the luminal side. The distal convoluted tubule was lined by tall dome shaped cells. Intertubular space is occupied by haemopoietic tissue. The cells were parencymatous and polygonal is shape.

Moreover splitting, shrinkage, liquification, hypertrophy, necrosis and vacuolation in various parts of kidney were also observed (Chatterjee et. al.,

PLATE NO. 3

Histology, histopathology and histochemistry of Kidney of control and experimental fish C. striatus (Bloch.)

Fig. 1 : T.S. of kidney of control fish stained with H - E X 800.

Fig. 2 : T.S. of kidney of fish exposed to 0.1 M phosphate for 72 hrs. stained with H - E X 800.

Fig. 3 : T.S. of kidney of control fish stained with PAS X 1120.

Fig. 4 : T.S. of kidney exposed to 0.1 M phosphate for 24 hrs. stained with PAS X 100.

Fig. 5 : T.S. of kidney of control fish stained with AB pH 2.5 – PAS X 1120.

Fig. 6 : T.S. of kidney of fish exposed to 0.05 M phosphate for 72 hrs. stained with AB-1 X 1120.

Fig. 7 : T.S. of liver of kidney fish stained with AB 1 – PAS X 100.

Fig. 8 : T.S. of kidney of exposed fish to 0.01 M stained with AB pH 2.5 – PAS X 400.

Abbreviations :

DCT - Distal convoluted tubule

PCT - Proximal convoluted tubule

L - Tubular Lumen

BC - Bowman Capsule

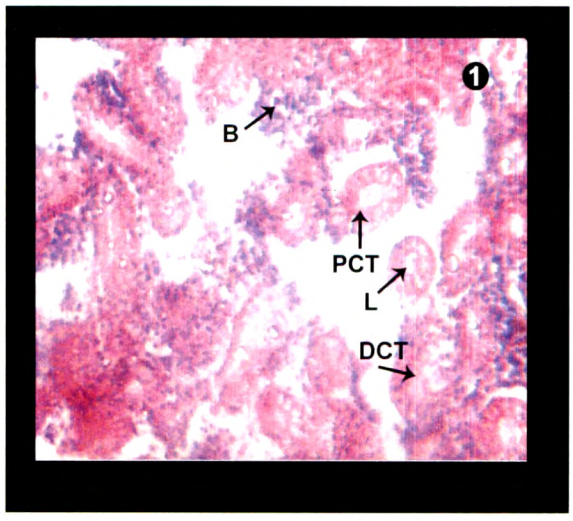
VC - Vaculated Cytoplasm

NTE - Necrosis of tubular epithelium

HEC - Hypertrophied epithelial cells

G - Glomerulus

PLATE NO. 3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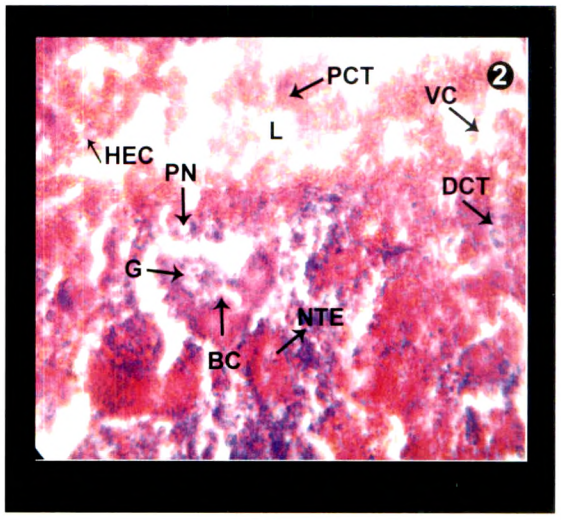
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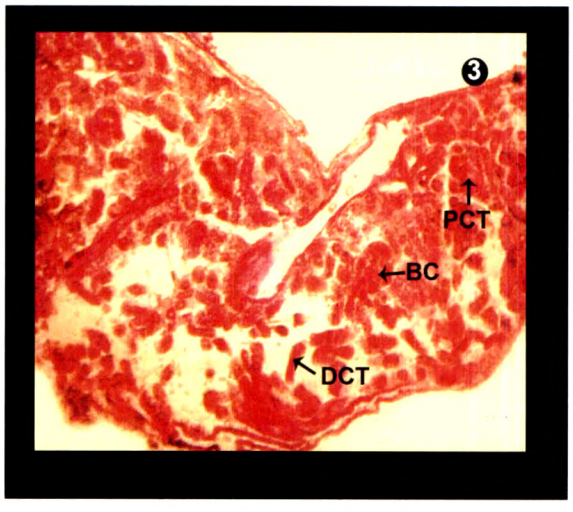
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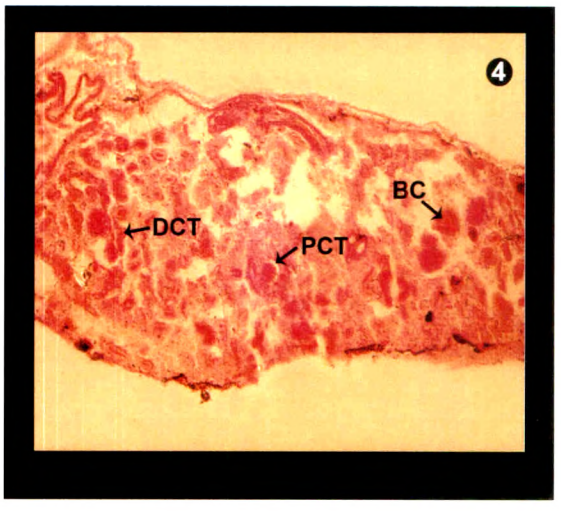
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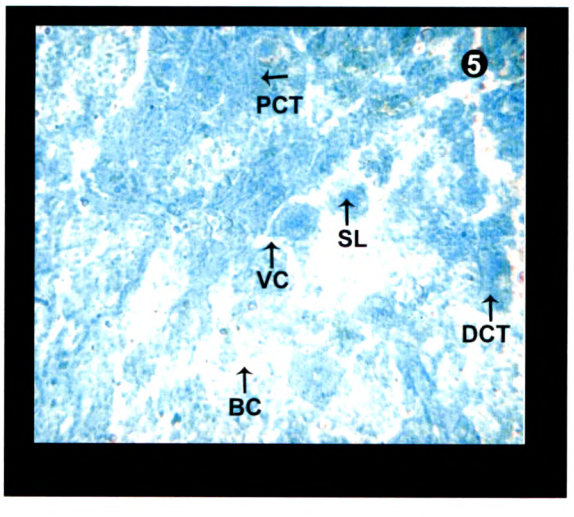
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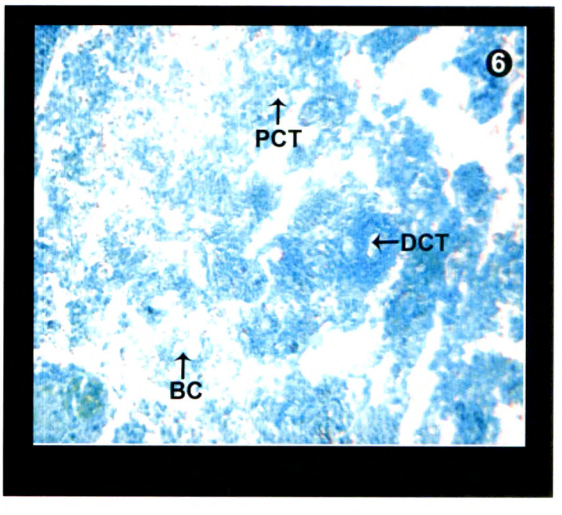
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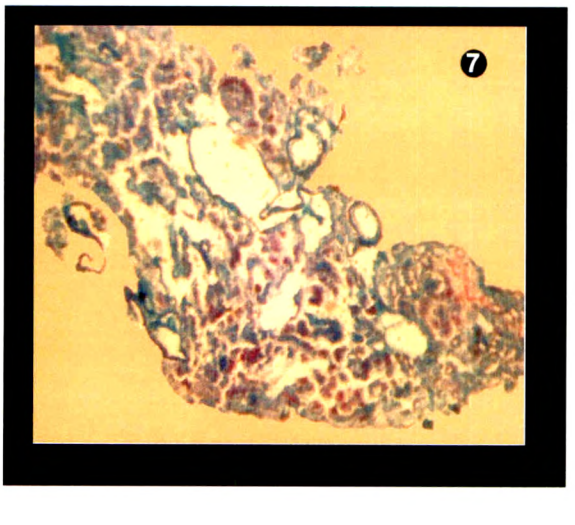
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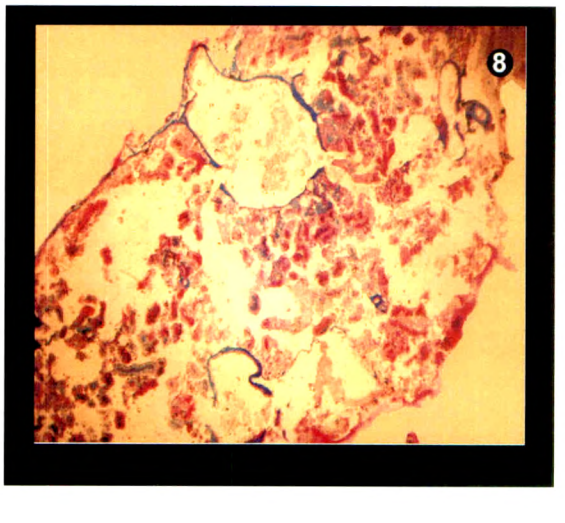
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1983 and Gupta and Dalela, 1987). Depletion of glycogen of kidney was recorded in Periophthalmus dipus when exposed to saline water (Bhan and Mansuri, 1978) and similar salinity effects on kidney glycogen of C. punctatus were reported by Bhatt et. al., (1979).

Mucus secreting segment in the nephron of Danio rerio and changes in it in different seasons were recorded by Bose and Chakraborty, (1979).

Fluoride induced histopathological study of kidney of Labeo rohita indicated extensive damage to the organ (Bhatnagar et. al., 2007).

From the survey of above literature study on the damage caused to piscine kidney due to phosphate is almost nil. Therefore present investigation was undertaken to determine the effects of phosphate toxicity on the histology, histopathology and mucia histochemistry of Channa striatus.

Histopathological observations in the kidney due to phosphate intoxication :

Histopathological alterations in the kidney because of phosphate intoxication are photomicrographically illustrated in the plate No. 3 Fig. 1 to 8. The important histopathological alteration are as follows.

0.006 M Phosphate :

The kidney of Channa striatus exposed to 0.006 M phosphate showed no marked changes in the histological architecture.

0.007 M Phosphate :

At 96 hrs. of exposure marked the beginning of the disintegration in the cells of PCT. The brush border lost its uniform structure. Haemopoietic tissue also get partially damage.

0.01 M Phosphate :

The kidney tissue showed thick lining of the Bowman's capsul shrunken glomerulus, increased capsular space with swelling (Plate No. 3, Fig. 2, 4, 6 and 8). The haemopoietic tissue appeared distinct empty spaces. Partial shrunken lumen was observed in renal tubules.

0.015 M Phosphate :

The glomeruli are shrunken and appeared as highly vacuolized structures due to destruction of cytoplasm and nuclei. All the proximal convoluted tubules lost their cellular material. Tubular epithelium showed necrotic changes. Lumen of the tubules of the tubules was invariably dialated. Thick Bowman's capsule, increased capsular spaces with swelling and sloughing off the epithelium of the capsule cell. The renal tubules showed vacuolated cytoplasm.

Histochemical observations on the control and exposed fish kidney to various concentrations of phosphate :

The histochemical observations on some important staining reactions employed in the present investigation of the kidney of Channa striatus are recorded in Table No. 6.1 according to the visually estimated staining intensity and shade with four plus (++++) representing the strongest activity. The distribution of mucosubstances in the kidney of control fish and intoxicated fishes are photomicrographically illustrated in Plate No. 3, Fig. 3 to 8.

Control fish :

Bowman's capsule, proximal tubule, distal tubule and haematopoietic tissue all stainly intense to moderate with PAS (Plate No. 3, Fig. 3, 5 and 7). Subsequent sequential staining indicated that Bowman's capsule and glomeruli contained only neutral mucosubstances proximal convoluted tubules

showed intense PAS reactivity sequential staining indicated presence of both neutral and acidic mucins. Distal tubules moderately stained with PAS but sequential staining techniques indicated simultaneous occurrence of neutral and acidic mucins. Haemopoietic tissue stained intensely with PAS but negatively stained with ABpH-1 and ABpH-2.5 indicating absence of acidic mucosubstances.

Histochemical alterations in the mucosubstance in the kidney due to phosphate intoxication :

Over all pattern of mucosubstances indicated that Bowman's capsule contain neutral mucins, which was increased in progressively higher concentrations of phosphate and duration of exposure.

Similarly proximal tubules with neutral and acidic mucins, distal tubules also have neutral and acidic mucins whereas haemopoietic tissue is without acidic mucosubstances.

In all cases progressive increase in concentration of phosphate and duration of intoxication resulted in the increase of mucosubstances.

Table No. 6.1

Histochemical observations on mucosubstances in the kidney of freshwater fish Channa striatus (Bloch.)

Sr. No.	Histochemical Techniques	Conc. of Phosphate	Generalized reactivities considering 24, 48, 72 and 96 hrs taken together			
			Bowman's Capsul	Proximal Tubule	Distal Tubule	Haematopoietic tissue
1	PAS	C	++++P	+++P	++P	+++P
		0.006 M	++++P	+++P	++P	+++P
		0.007 M	++++P	+++P	++P	+++P
		0.01 M	++++P	+++P	++P	+++P
		0.015 M	++++P	+++P	+++P	+++P
2	AB pH 1	C	-	-	+B	-
		0.006 M	-	-	+B	-
		0.007 M	-	-	+B	-
		0.01 M	-	-	++B	-
		0.015 M	-	-	+++B	-
3	AB pH 1 - PAS	C	-	+++P	++BP	+++P
		0.006 M	++++P	+++P	++BP	+++P
		0.007 M	++++P	+++P	++BP	+++P
		0.01 M	++++P	+++P	++BP	+++P
		0.015 M	++++P	+++P	+++BP	+++P
4	AB pH 2.5	C	-	+++B	++B	-
		0.006 M	-	+++B	++B	-
		0.007 M	-	+++B	++B	-
		0.01 M	-	+++B	++B	-
		0.015 M	-	+++B	+++B	-
5	AB pH 2.5 - PAS	C	++++P	+++B	++BP	+++P
		0.006 M	++++P	+++B	++BP	+++P
		0.007 M	++++P	+++B	++BP	+++P
		0.01 M	++++P	+++B	+++BP	+++P
		0.015 M	++++P	+++B	+++BP	+++P

Discussion :

It has frequently been observed that acute or chronic treatment of pesticides cause histopathological and biochemical alterations in kidney because it is involved in detoxification mechanism. The phenolic compounds are maximaly accumulated in the Kidney (Llyod and Swift, 1976).

Fluoride induced histopathology of kidney of teleost fish is studied by Bhatnagar et. al., (2007) showed following observations massive doses of induce tubular necrosis, especially in the convoluted portion and inflammation of glomerules leading to impaired kidney function.

Gupta and Dalela (1987) reported histological changes in kidney of Notopterus notopterus, exhibiting degeneration and dissolution of epithelial cells of renal tubules, hypertrophy and necrosis following sublethal exposure to phenolic compounds. Similar observations were made by Csepai, (1978) in Cyprinus carpio chronically exposed to Anthio 40 EC, Satox and Basden 10G. Histopathology of carp Labeo rohita hexachlorocyclohexane indicated most of the alterations occurred in tubular cells rather than glomerulus (Das and Mukherjee, 2000).

Histology :

Histological architecture of kidney of controlfish Channa striatus showed regular structure similar to other teleostean fishes. It showed nephrons showing Bowman's capsule and glomerulus. Bowman's capsule is formed of

inner visceral cells and outer parietal layer of cuboidal cells. Proximal convoluted tubules characterized by columnar cells with brush border. The distal convoluted tubule lined by tall dome shaped cells. Intertubular space is occupied by haemopoietic tissue which is similar with other teleostean fishes.

Histopathology :

The phosphate more than 2 mg/l in open water indicates organic pollution (Pomeroy, et. al., 1965). Once absorbed, toxicant is transported by blood circulation to liver for transformation or storage and if transformed in the liver it may be excreted through kidney (Lindstoma, Seppa et. al. 1981).

In the present investigation histopathological alterations were distinct progressively to higher concentrations of phosphate for a prolonged period of time. At 0.007 M phosphate proximal convoluted tubule get affected, inner brush border lost it's uniformness and haemopoietic tissue also get partially damaged. In 0.01 M phosphate exposure lining of Bowman's capsule becomes thick, glomerulus shrunkened. The haemopoietic tissue appeared as a mass of detached cells with distinct empty spaces. Lumen of renal tubule were partially shrunken. At highest concentration of phosphate glomeruli become highly vacuolized structures, proximal convoluted tubules lost their cellular material. Tubular epithelium showed necrotic changes. Lumen of the tubule were invariably dialated. Thick bowman's capsule, increased capsular space with swelling and sloughing off the epithelium. The renal tubules showed vacuolated cytoplasm.

Similar observations were mode by Csepai in Cyprirus carpio chronically exposed to Anthio 40 E. C., Satox and organophosphate compounds.

Histopathological effects fluoride on the kidney of teleost fish Labeo rohita indicated massive doses of phosphate induces tubular necrosis, inflammation of glomerulus leading to impaired kidney functions like polyurea (Bhatnagar et. al., 2007).

The histopathological alterations in the present investigation match with earlier observation on fish toxicity made by Csepai (1978) and Bhatnagar et. al., (2007).

Severely shrunkan lumen of tubules is also suggestive of hindered tubular reabsorption.

Histochemisty :

In the present investigation Bowman's capsule showed present of neutral mucosubstances, proximal tubules and distal tubules contain neutral and acidic mucins; where as haemppoietic tissue is without acidic mucosubstances and contain only neutral mucosubstances.

The progressive exposure of the kidney of C. straitus to higher doses of phosphate and for prolonged periods up to 96 hrs. showed marked increase in the mucosubstances in all types of tissues of kidney.

Longley et. al., (1963) has directed the role of mucoid substance as a protective or hydrophilic colloids in preventing the precipitation of solutes preventing the stone formation. There is possible role of mucubstances in the present study might be related to osmoregulatory function of the kidney. Increase in acidic mucins seem to be related with the protective function by binding with toxicant. Such a role of sialic acids in binding with salts has been described in T.

mossambica larvae by Jirge (1971). Bose and Chakraborty (1979) reported the acidic mucins in kidney of D. rerio are related with the protective role during cellular injury.