Chapter – IV

Histological, Histopathological and Histochemical Observation and Discussion on Stomach of Channa striatus (Bloch.) Exposed to various Concentration of Phosphate

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Stomach :

Review of literature :

The existing literature on the stomach of fishes showed that there are several histological, morphological and anatomical studies (Weisel, 1973, Martin and Blaber, 1984). Gastric glands are preset in the cardiac stomach and muscular organization changed in the stomach. The histological structure of stomach of Clarius batrachus had been comprehensively given by Khanna (1996). According to Khanna the stomach has thicker muscular wall and is lined by columnar epithelium raised into several primary and secondary folds. Numerous gastric glands present below the epithelium.

Toxicological studies such as accumulation of Ca and Cd in the intestinal wall of exposed fishes Tilapia was shown by (Pelgrom et.al., 1995). Bhatnagar et. al., (2007) studied fluoride induced histopathological changes in the intestine of fresh water teleost, Labeo rohita.

According to Martin and Blaber, 1984 some teleosts were without stomach and some contain stomach which could be differentiated into cardic and pyloric regions. Gastric glands were present in cardiac stomach, but were absent in the pylorus. Muscularis organization changed in the stomach region which showed outer longitudinal and inner circular layer of fibres.

Jirge (1970), Suganuma et. al., (1981b) and Jadhav (1985) have given detailed histochemical account on stomach mucosubstances on some teleostean fish species. Biochemical work on pH changes in stomach in relation to temperature, salinity combination has been reported by Katre (1975).

Histopathological changes to acute and chronic pH level in the water (Daye and Garside, 1976), due to thiodan 35 E. C. and Agallol (Amminikutty and Rege, 1978), due to urea toxicity (Srivastava and Srivastava 1979b) and due to aldrin (Ratnakar and Awasthy, 1979) have been reported. Chattergy et. al., (1983) have found rupturing of gastric mucosa, loss of cellular outline of epithelial cells, mesh like muscularis and necrosis in muscular and connective tissues in H. fossils due to exposure to 3 – 10 ppm phenol.

In present investigation phosphate toxicity had been studied for histology, histopathology and histochemistry of mucins in the stomach of C. striatus have been determined.

1) Histological observation on the control fish stomach :

The histological observations on the stomach of control fish C. striatus in the present investigation showed following histological feature.

Innermost layer i.e. mucusa is lined by columnar epithelium raised into several primary and secondary folds forming rugea. Numerous gastric glands of simple tubular type are observed below the epithelium (Plate No. 1, Fig. 1, 2) and open into the lumen of stomach. The epithelium rich in mucous cells. Depending on the nature of secretion mucous cells may of different types. Gastric glands contained low columnar cells and there were no mucous cells. Gastric glands formed many layered structure (Plate No. 1, Fig. 2). Mucosa is followed by lamina propria and submucosa. Lamina propria formed a small layer of connective tissue below columnar epithelium and gastric glands where as submucosa formed a thick layer of connective tissue. Both lamina propria and submucosa was highly vascularized. Submucosa was followed by inner layer of circular muscles and other layer of longitudinal muscles. The outermost layer of serosa was characterized by a layer of loose connective tissue which was covered by simple cuboidal mesothelium (Plate No. 1, Fig. 1 and 2).

PLATE NO. 1

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

Fig. 1 : T.S. of Stomach of control fish stained with H - E X 100

Fig. 2 : T.S. of stomach of fish exposed to 0.006 M phosphate for 48 hrs H - E X 100.

Fig. 3 : T.S. of stomach of control fish stained with AB pH 2.5 X 100

Fig. 4 : T.S. of stomach of fish exposed to 0.05 M phosphate for 48 hrs stained with AB pH 2.5 X 100.

Fig. 5 : T.S. of control fish stained with Nor-AB 2.5 – PAS X 100.

Fig. 6 : T. S. of stomach of fish exposed to 0.006 M phosphate stained with AB pH 2.5 PAS X 400.

Fig. 7 : T. S. of control fish stained with PAS X 100

Fig. 8 : T. S. of stomach of fish exposed to 0.1 M Phosphate stained with AB pH-1-PAS X 100

Abbreviations :

SM - Submucosa

M - Mucosa

MC - Mucous Cells

S - Serosa

LM - Longitudinal muscularis layer

F - Flattened rugae

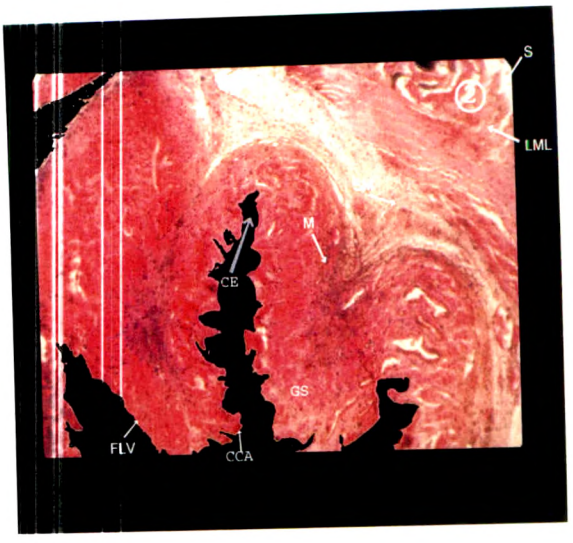
GS - Gastric glands

F - Fusion of cells

CCA - Cracked clay appearance

PLATE NO. 1


1
FV
M
FLV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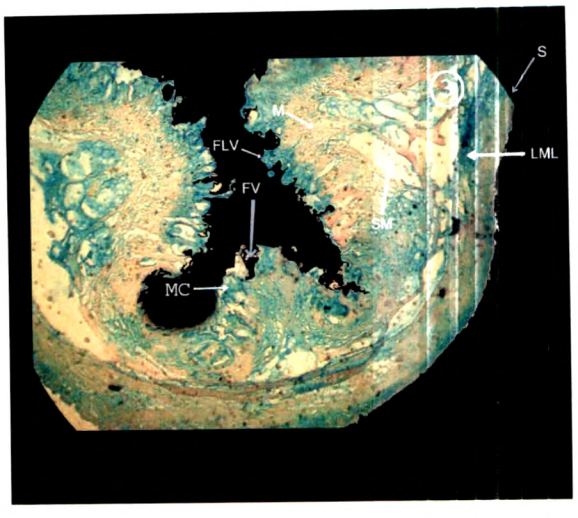
S
LML
M
CE
GS
FLV
CCA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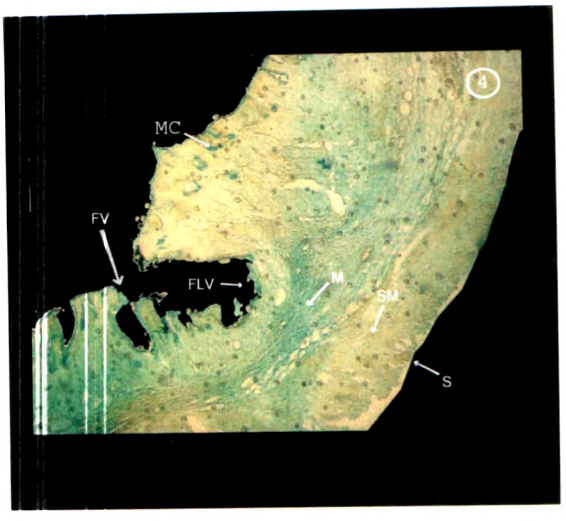
S
M
FLV
LML
FV
SM
MC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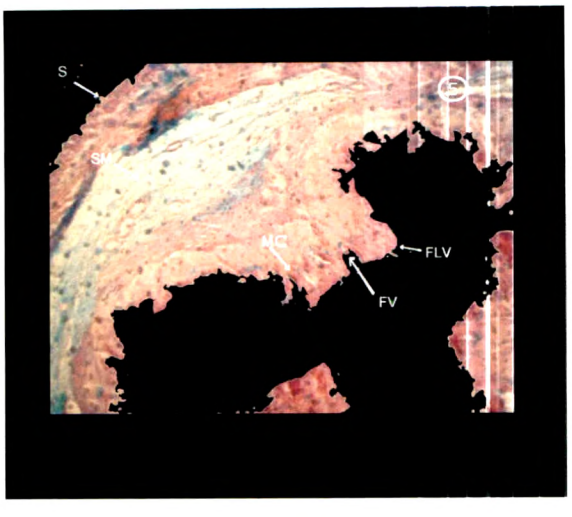
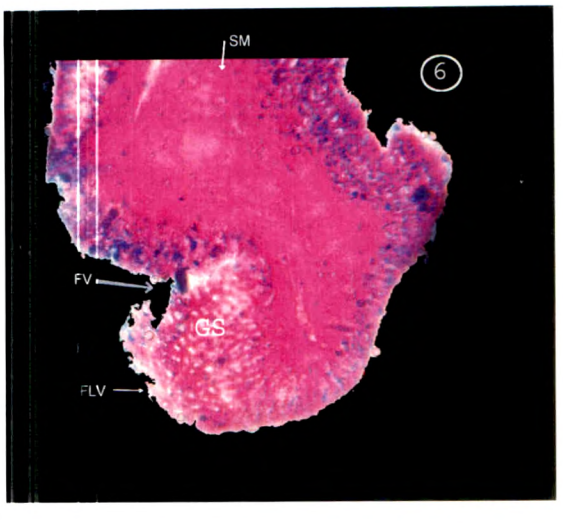
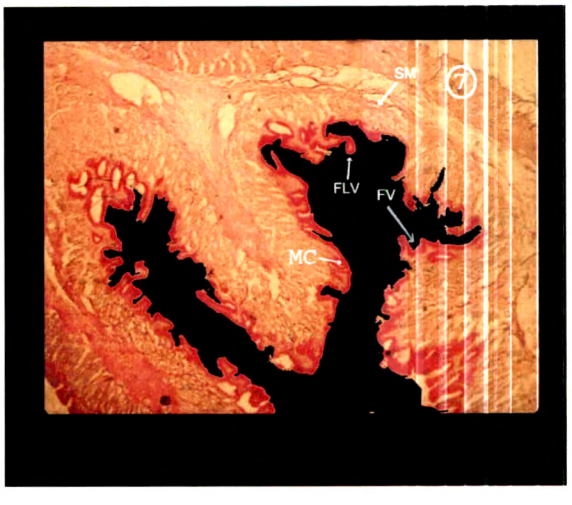
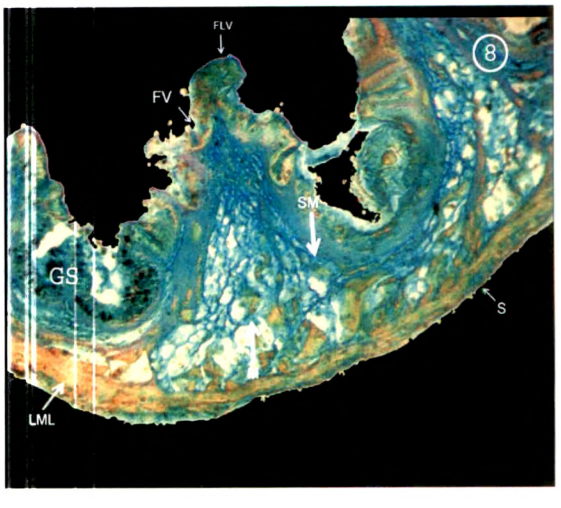
FV
FLV
M
SM
S


5
S
SM
MC
FLV
FV


SM
6
FV
GS
FLV


SM
7
FLV
FV
MC


FLV
8
FV
SM
GS
S
LML

2) Histopathological observations on the stomach of phosphate intoxicated fish :

The histopathological changes in the various tissues of stomach during phosphate intoxication at different concentrations are photomicrographically illustrated in Plate No. 1, Fig. 2, 4, 6 and 8.

In exposed group a degenerative effect was evident in the mucosal lining. Hypertrophy of epithelial cells, swelling or oedema of lamina propria and fusion of glands. Flattening of mucosa giving cracked clay appearance was also evident.

i) 0.006 M Phosphate :

The surface mucosa cells were slightly enlarged and nuclei had lost their shape. Gastric glands were stained intensely with HE. The rest of the architecture remained unaltered.

ii) 0.007 M Phosphate :

After 96 hrs of treatment stomach tissues were severally damaged Mucosal lining cells were affected and seen slightly separated from each other. The free margins of mucosal layer was damaged leading to exposure of gastric glands. Occasionally fusion in the rougae was also observed.

iii) 0.01 M Phosphate :

Mucosal cells were considerably swollen with enlarged nuclei. These cells became extensively vacuolated. The rugae extensively flattened. Hypertrophied epithelial cells were observed. The swelling and loosening of gastric glands were noticed. Submucosa showed marked degeneration.

iv) 0.015 Phosphate :

The mucosa was extensively damaged, rugae became flattened and there was sloughing off the mucosal lining at 48 hrs of exposure. Hypertrophy of epithelial cells, swelling or oedema of lamina propria and fusion of rugae.

Ruptured mucosa flattened columnar epithelial cells and a cracked clay appearance was apparent (Plate No. 1, Fig. 2).

Histochemical observations on the control and phosphate intoxicated fishes :

The histochemical observations on some important staining reactions used in the present investigation of stomach of C. striatus are recorded in Table No. 4.1 according to the visually estimated staining intensity and shade with four plus (++++) representing the strongest activity. The distribution of mucosubstances in the control and phosphate intoxicated C. striatus are photomicrographically illustrated in Plate No. 1, Fig. 2 to 8.

On the elaboration of mucosubstances the surface mucous cells of the stomach could be differentiated into three types and oxyntopeptic cells in the gastric glands. All these four types of cells showed distinct responses during phosphate intoxication.

Surface mucous cells :

The surface mucous cells in the stomach of C. striatus reacted intensely towards PAS. The surface mucous cells can be differentiated surface mucous cells can be differentiated as M_1 cells produce neutral mucosubstances, M_2 contained sulfo mucins in them and M_3 cells contained carboxymucins.

In the intoxicated fish it is observed that at higher concentrations of phosphate and to the prolonged exposure up to 96 hrs show more production of mucins by all surface mucous cells.

Oxyntocopeptic :

These cells exhibited poor PAS staining (Plate No. 2, Fig. 3 to 8). The rest of the staining reactivities are similar to M_1 indicating the presence of only neutral mucosubstances in them.

In general all the mucous producing cell during exposure with various phosphate concentrations show progressive increase in the production of mucous.

Table No. 4.1

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

Sr. No.	Histochemical Techniques	Concentration of phosphate	Generalized reactivities considering 24, 48, 72 and 96 hrs. taken together			Gastric Gland Oxyntico peptic cells
			M1	M2	M3	48 hrs
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	-
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	++B	++P	++P
		0.006 M	++++P	++B	++P	++P
		0.007 M	++++P	++B	++P	++P
		0.01 M	++++P	++B	++P	+++P
		0.015 M	++++P	+++B	+++P	+++P
4	AB pH 2.5	C	-	-	++B	-
		0.006 M	-	-	++B	-
		0.007 M	-	-	++B	-
		0.01 M	-	-	++B	-
		0.015 M	-	-	+++B	-
5	AB pH 2.5 - 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

Discussion :

Histology :

The histological studies in the present investigation on stomach of control C. striatus showed that innermost mucosa is lined by columnar epithelium raised into several primary and secondary folds forming rugae. Numerous gastric glands of simple tubular type are observed below the epithelium and open in the lumen of stomach. The epithelium is rich in mucous cells. Gastric glands contained low columnar cells devoid of mucous cells. Gastric glands formed many layered structure. Mucosa is followed by lamina propria, submucosa, muscularis and serosa. Both lamina propria and submucosa were highly vasuclarized.

More or less similar histological structure of stomach has been descried in other fishes (Pasha, 1964, Jirge, 1970, Weisel, 1973, Reifel and Travilli, 1978, Martin and Blaber, 1984).

Stirling (1984) was of the opinion that the surface mucous cells in teleostean fishes might be secreting HCl and mucous also. He also conducted that surface mucous cells are more closely resemble with the secretary cells in the gastric glands of amphibians. Secreting both HCl and enzymes (pepsinogen), which is also described by Sedar (1962). Ezeasor (1981) described the fine structure of surface mucous cells, oxyntic cells and endocrine cells in the gastric mucosa of S. gairdneri. In the present investigation also single type of cells were observed in the gastric glands which resemble to the oxyntocopeptic cells in other fishes histopathology.

The observations pertaining to effect of phosphate on the stomach of C. striatus in the present study are in agreement with earlier reports on stomach of different fish species after exposure to fish species (Shashtry and Malik, 1979,

Bakthavathsalam, et. al., 1982, Sinha, 1996), Metals (Shastry and Gupta, 1978; Natrajan, 1979, Naidu et. al., 1983; Pandey et. al., 1994, Srivastava, 2007) and dyes (Srivastava et. al., 1998).

The histopathology changes in the stomach of C. striatus in the present study were observed at acute, sublethal levels of phosphate. The histopathological changes include degenerative effect proliferation, disintegration and necrosis of mucosa, submucosa and serosa. The rugae tend to become flattened and there is sloughing off the mucosal lining. Hypertrophy of surface epithelial cells, swelling or oedema of lamina propria and fusion of stomach folds due to excessive hypertrophies, ultimately leading to rupture of surface epithelial lining was evident at higher concentrations of phosphate. Flattened of folds and a cracked clay appearance was also noticed.

Shastry and Malik (1979) reported degeneration of mucosal epithelium and hyperactivity of mucous secreting cells in dimecron treated C. punctatus. Ruhela, et. al., (2007) reported pathological changes in Clarius batrachus infected with procamallanus. Similar effects were observed in the intestine of Barbus stigma when exposed to different concentrations of nitrates (Natarajan, 1979).

Thus the observations recorded in the present study are in close agreement with the above reports.

Histochemistry :

Very few investigations have studied distribution, localization and characterization of mucosubstance in teleostean fishes (Jirge, 1970; Reifel and Travill, 1978, 1985). Jadhav (1985) has found only neutral and sulfomucins in the surface mucous cells in T. mussambica. Elbal and Agulleiro (1986) described that

the gastric mucosa of Sparus auratus contained neutral mucins and sulfomucins in numerous simple tubular glands and dark cells. In M. gulio only neutral mucins have been observed in gastric glands (Sultana and Rao, 1986).

In the present investigation surface mucous cells contain at least three types cells M1, M2 an M3 elaborating neutral, sulpho and carboxy mucins respectively. The secretion of all these cells was found to be progressively increased with higher concentration of phosphate coupled with prolonged treatment. Induced mucosubstances might be playing role in detoxification process. Mitjavila et. al., (1968) and Fox (1979) also attributed protective role to the mucous in intestine of mammals.