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Heavy metal induced histopathological alterations in liver, muscle and kidney of freshwater cyprinid, *Labeo rohita* (Hamilton)

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Abstract
In the present investigation, adult live specimens of edible freshwater cyprinid, *Labeo rohita* were procured from local fish market at Hambran road, Ludhiana, Punjab from December 2016 to June 2017 and various fish tissues (liver, muscle and kidney) were processed for heavy metal estimation and histopathological studies. The observations on histopathology included several hepatic lesions *viz*. cytoplasmic degeneration, severe necrosis, melano-macrophage centres, infiltration of leukocytes, pyknosis and nuclear degeneration. Shortening and elongation of muscle bundles were well-marked in muscle tissue. Renal alterations included edema, irregular diameters, degeneration and atrophy of renal tubules. The results reflected that the content of toxic heavy metals (arsenic, chromium, cadmium, manganese and lead), which were beyond the permissible limits (WHO/FAO), might have led to variations in histo-architecture of vital organs i.e. liver and kidney. However, the content of chromium and manganese exceeded the prescribed limits in muscle tissue which might have caused the histological alterations.

Keywords: heavy metals, *Labeo rohita*, Liver, kidney, muscle, histopathology

1. Introduction
Contamination of aquatic bodies with a vast array of pollutants has seriously increased the worldwide attention. Anthropogenic activities resulting from modern agricultural practices, rapid urbanization and industrialization involve the increased release of various chemical pollutants and toxicants, such as industrial effluents, biocides, pesticides, heavy metals etc. which ultimately reach the aquatic environments and become responsible for their degradation [1, 2]. Among these pollutants, heavy metals have been recognized as strong biological poisons because of their persistent nature, toxicity, tendency to get accumulated in organisms and undergo biomagnification [3, 4].

The term 'heavy metal' refers to any metal or metalloid that has relative atomic density greater than 4g/cm³ or 5g/cm³ and is toxic even at very low concentrations [5, 6]. Heavy metals are environmentally ubiquitous. They get easily dissolved and are transported by water to be readily taken up by aquatic biota. Due to its high toxicity, long persistence and non-biodegradable nature in the food chain, they constitute a core group of aquatic contaminants causing cellular toxicity, mutagenicity and carcinogenicity in animals [7, 8].

Among the aquatic fauna, fishes are the valuable organisms in the study of heavy metal pollution, because they explore freely among the different trophic levels in an aquatic environment [9, 10]. Fish fauna assimilate heavy metal contaminants by different ways like intake of particulate matter suspended in water, ion-exchange of dissolved heavy metals across lipophilic membranes (gills) and adsorption on surface of tissues and membrane. Heavy metal distribution among different fish tissues also depends upon the mode of exposure (dietary or aqueous) [11].

Histopathological changes are used as biomarkers to evaluate the overall health of fish exposed to contaminants [12]. The major advantage of using such markers in environmental monitoring is that it allows examining specific vital organs including liver, gills and kidney that are responsible for fundamental functions such as accumulation and biotransformation of xenobiotics, excretion and respiration in fish [13]. Liver is associated with the detoxification and biotransformation due to its position, function and blood supply and also, it is one of the organs most vulnerable to damage caused by variety of toxicants [14]. Kidney is an important

organ for the maintenance of homeostasis with respect to water and salts, metabolic waste excretion from blood and partially for the metabolism of xenobiotics [15, 16]. Basically, liver and kidney are considered as the crucial organs suitable for histopathological examination in order to study the damage to cells and tissues [17, 18]. Degenerative changes in muscle tissue have also been noted as sign of exposure to environmental toxicants like heavy metals or pesticides [19, 20]. Therefore, in the present study, heavy metal induced histopathological alterations were examined in different tissues (liver, muscle and kidney) of edible and commercially important cyprinid fish, *L. rohita* (Hamilton) procured from local fish vendors of Ludhiana, Punjab *vis-a-vis* cultured fish.

2. Material and Methods

2.1 Procurement of the fish

Live specimens of adult freshwater cyprinid, *Labeo rohita*, irrespective of size, weight and sex were procured from local fish market at Hambran road in Ludhiana city (30°56´N; 75°52´S) from December 2016 to June 2017. Source of captured fish, as told by fish vendors, was from different districts of Punjab State (India) and local fish ponds of Ludhiana city and Sutlej River. Similarly, control fish i.e. *Labeo rohita* was procured from fish culture ponds, College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana.

2.2 Analysis of heavy metals

Heavy metals in fish were estimated using Inductively Coupled Argon Plasma Atomic Emission Spectrophotometer (ICAP-AES, Thermo iCAP-6300) as recommended by Yousafzai *et al* [21]. The samples (liver, muscle and kidney) were processed for the estimation of eight heavy metals i.e. arsenic, cadmium, chromium, copper, manganese, nickel, lead and zinc.

2.3 Histopathological studies

Adult fish samples were dissected and different tissues (liver, muscle and kidney) were removed, excised of fat and adhering tissues. The representative samples were carefully preserved in labeled sampled bottles containing aqueous Bouin's fixative for 24 hours.

2.3.1 Tissue processing, sectioning and staining

After complete fixation in aqueous Bouin's solution for 24 hours, the tissues were dehydrated in ascending series of ethanol, cleared in xylene and embedded in paraffin wax (melting point between 58-60 °C). 4-6µm thick sections were cut with the help of rotary microtome.

After usual de-waxing with xylene followed by rehydration in descending series of ethanol, the sections were stained in haematoxylin, counterstained with eosin, dehydrated in ascending ethanol series, cleared in xylene and mounted in DPX [22]. The slides were observed under compound light microscope at 100X and 400X magnification and photomicrographs were taken using digital camera (Olympus CH20i). Changes in various tissues of cyprinid were observed and compared with the control fish for the determination of histological alterations.

3. Results and Discussion

3.1 Histopathology of liver

In the present study, the liver of control fish showed typical compact histo-architecture which was characterized by normal hepatocytes containing granular cytoplasm and nuclei and presence of sinusoids as shown in Fig. 1A, B. On the contrary, sections of liver of market cyprinid revealed various alterations such as, cytoplasmic degeneration, severe cell death, melano-macrophage centres (Fig. 1C), presence of pyknotic nuclei, mild necrosis (Fig. 1D), nuclear degeneration, hyper-vacuolization. Infiltration of leukocytes in sinusoids were also noticed (Fig. 1E). Disruption of normal hepatocytes in addition to hemorrhage, vacuolization of hepatocytes, rupture of blood vessels (Fig. 1F) were found to be the well-marked changes in histology of liver. The alterations in histo-architecture of liver were probably due to the accumulation of heavy metals i.e. arsenic (1.85 ppm), cadmium (2.43 ppm), chromium (1.71 ppm), lead (9.66 ppm) and zinc (58.02 ppm) beyond the certified limits as shown in Table 1.

Previous studies reported that the histological changes in the liver were not metal specific but due to the response of toxicants in the fish body [23]. Sorenson *et al.* [24] derived a link between metal exposure and lesions in liver. Our results are well supported by the work of Saini [25] who recorded severe histopathological lesions such as infiltration of lympho-nuclear cells, degeneration of hepatic parenchyma and deformation of hepatocytes, induced by heavy metals, in liver of *L. rohita* caught from natural freshwaters of Punjab. Under laboratory conditions, Chavan and Muley [26] exposed *Cirrhina mrigala* to sub-lethal concentration of lead acetate and examined several pathological changes in liver tissue such as cytoplasmic vacuolation, congestion in sinusoids and venules and focal necrosis. Likewise, Jalaludeen *et al.* [27] noticed many prominent alterations in hepatic tissue of *Tilapia mossambica* which included severe damage, marked proliferation of ducted cells, conversion of liver tissue into sponge mass and large vacuoles after exposure to cadmium sulphate. Patnaik *et al.* [28] too, exposed *Cyprinus carpio* to the sub-lethal concentration of lead and cadmium and found hypertrophy of hepatocytes, vacuolation and metal accumulation in lead treated liver. However, pyknotic nuclei, severe necrosis and vacuolization were well marked in cadmium treated liver. Complete disintegration of liver tissue and marked necrosis was detected by Mary *et al.* [29] in *C. mrigala* following exposure to lead nitrate.

Similarly, liver lesions were observed in *C. gariepinus* and *Mugil capito* inhabiting Manzalah lake (Egypt) which included degeneration, necrosis in hepatocytes, hemosidrin in liver of *C. gariepinus* whereas fatty degeneration, hemolysis, hemorrhage, edema and congestion of blood sinusoids were noted in *M. capito* [30]. Histopathological alterations in the liver of both the fish species might be due to the direct result of fertilizers, salts and sewage, which entered the lake with the drainage water as noted by Tayel *et al.* [31] who recorded similar histological variations in liver of *Mugil* species living in same lake.

Table 1: Level of heavy metals in different tissues of market cyprinid fish with respect to control fish

Tissues / Elements	Liver		Muscle		Kidney		Certified limits (WHO/FAO)
	Control	Market	Control	Market	Control	Market	
Arsenic	0.64±0.11	1.85±0.21*	0.68±0.12	0.55±0.15	0.35±0.11	1.49±0.15*	1.00
Cadmium	0.77±0.074	2.43±0.25*	0.069±0.02	0.12±0.04	0.55±0.12	1.71±0.15*	1.00
Chromium	0.027±0.07	1.71±0.14*	0.025±0.005	1.31±0.28*	0.02±0.007	1.86±0.21*	0.05
Copper	5.21±0.76	4.07±0.74	2.65±0.34	2.87±0.65	1.71±0.13	4.26±0.49	10.00
Manganese	0.21±0.06	2.86±0.17*	0.32±0.05	2.16±0.19*	0.26±0.07	2.67±0.24	5.00
Nickel	0.305±0.05	1.42±0.16	0.28±0.06	0.26±0.07	0.29±0.03	0.55±0.15	80.00
Lead	3.84±0.26	9.66±1.46*	3.55±0.21	4.32±0.16	3.42±0.40	8.06±0.64*	5.00
Zinc	39.99±1.66	58.02±4.05*	34.34±2.14	36.29±3.17	33.41±4.64	54.55±5.31*	50.00

Values are Mean±SE

Values with * (in a column) reveal significant difference at $p \leq 0.05$ for each tissue of market fish with respect to control fish

3.2 Histopathology of muscle

Histological study of muscle tissue of the control cyprinid showed various layers i.e. epidermis, dermis, myo-epithelium and normal myotomes with equally spaced muscle bundles which indicated the fish to be in unstressed conditions (Fig. 2A, 2B). In contrast, muscle tissue of market cyprinid (*L. rohita*) exhibited prominent changes like shortening of muscle bundles, edema and necrosis (Fig. 2C, 2D). Elongation of muscle bundles was also observed (Fig. 2E, 2F).

The results indicate that the alterations in muscle tissue of *L. rohita* might have occurred due to significantly high concentration of chromium (1.31 ppm) and manganese (2.16 ppm) and their accumulation beyond the prescribed limit in the muscle tissue (Table 1). Although, muscle is the most edible part of fish body but it is also the tissue which is in close contact with pollutants dissolved in water [32, 33]. According to Saad *et al.* [34], if fish inhabiting polluted water displayed epithelial lesions in muscle tissue then that would most probably be invaded by micro-organisms which might cause severe epidermal pathology, resulting in degeneration of muscle bundles.

The results of present study on market carp are corroborated by the findings of Abbas and Ali [35], who noted several histological variations such as destruction and vacuolation in the muscle cells of *Oreochromis* species, following exposure to chromium. Likewise, Patnaik *et al.* [28] studied the histology of *C. carpio* exposed to sub-lethal concentrations of lead cadmium. The authors reported marked thickening and separation of muscle bundles with intracellular edema. Similarly, degeneration of muscle bundles along with the aggregation of inflammatory cells between them, focal areas of necrosis, vacuolar degeneration in muscle bundles and atrophy of muscle bundles have been reported in fish exposed to different pollutants [36]. Several histopathological alterations were also induced by Cauvery river pollutants in muscle tissue of *L. rohita* which included shortening of muscle bundles, severe intra muscular edema and necrosis of muscle bundles. All these changes indicated the fish under highly stressful conditions due to more polluted region receiving effluents from industrial complex [37].

3.3 Histopathology of kidney

Kidney is a vital organ of excretion and osmoregulation and it also helps in maintaining the homeostasis. It is also responsible for selective reabsorbtion, which helps in maintaining volume and pH of blood and body fluids and erythropoieses [38].In the control fish, sections of kidney revealed normal architecture which consists of Bowman's capsule closely arranged in renal tubules (Fig. 3A, 3B).

However, histology of kidney of market cyprinid exhibited atrophy of renal tubules, aggregation of inflammatory cells, loss of cellular integrity of renal tubules and degeneration of renal tubules (Fig. 3C, 3D). Various alterations such as irregular diameters of renal tubules, few necrotic areas and infiltration of edematous fluid between renal tubules were also noted (Fig. 3E, 3F). The histopathogical alterations observed in the present study may be correlated with high content of heavy metals such as arsenic (1.49 ppm), cadmium (1.71 ppm), chromium (1.86 ppm), lead (8.06 ppm) and zinc (54.55 ppm) and its accumulation in the kidney tissue beyond the prescribed limit by WHO/FAO as depicted in Table 1.

Similar histopathological changes i.e. enlargement of renal tubules, desquamation of epithelial lining, hypertrophied nuclei, edema, dilation of renal tubules, severe necrosis, pyknotic nuclei, vacuolization, disorganized blood capillaries in glomerulus were induced in kidney of *Channa punctatus* following exposure to sub-lethal concentration of zinc [39]. The authors suggested that high accumulation of zinc in kidney would probably dysfunction the detoxification mechanism of renal tissue and cause histopathological abnormalities in it. Various alterations such as mild edema, reduction in cell size, severe damage and further degeneration of renal tubules, disorganization of renal tissue and necrosis were noticed when *T. mossambica* was exposed to sub-lethal concentration of cadmium sulphate for 20 days under laboratory conditions [27]. Likewise, Rana *et al.* [40] noted various changes such as aggregation of inflammatory cells, dilation in capillary tubes of renal tubules and hemolysis in kidney of *C. carpio*, following exposure to chromium at sub-lethal concentrations.

Our results are in accordance with the findings of Saini. [25] who noticed histological changes such as, vacuolar degeneration, congestion of blood vessels and degeneration of tubules in the kidney of fish *L. rohita* procured from from freshwaters of Punjab. Dhevakrishnan and Zaman. [37] too, studied the effect of Cauvery river pollutants on kidney tissue of freshwater fish *L. rohita* and noted various alterations such as degeneration and atrophy of renal tubules, degeneration in glomerulus, disorganization of glomerulus, severe necrosis and highly pyknotic nuclei following exposure to untreated industrial effluents which were discharged in the water body. Several renal lesions in the kidney of *Mugil cephalus* and *Mugil capito* from Lake Manzala, Egypt as a result of water quality changes have also been reported by Tayel *et al.* [31] which included degeneration, necrosis, fibrosis, hemorrhage and hemosiderosis.

4. Conclusion

In conclusion, severe histopathological lesions and cellular alterations were observed in major target organs (liver, kidney and muscle) of market fish which could be attributed to the significant accumulation of several heavy metals in these tissues beyond the prescribed limits of WHO/FAO. Hence, there is a need of stringent check on the industrial effluents

being discharged in freshwater bodies so as to avoid the influx and accumulation of such toxicants in aquatic fauna.

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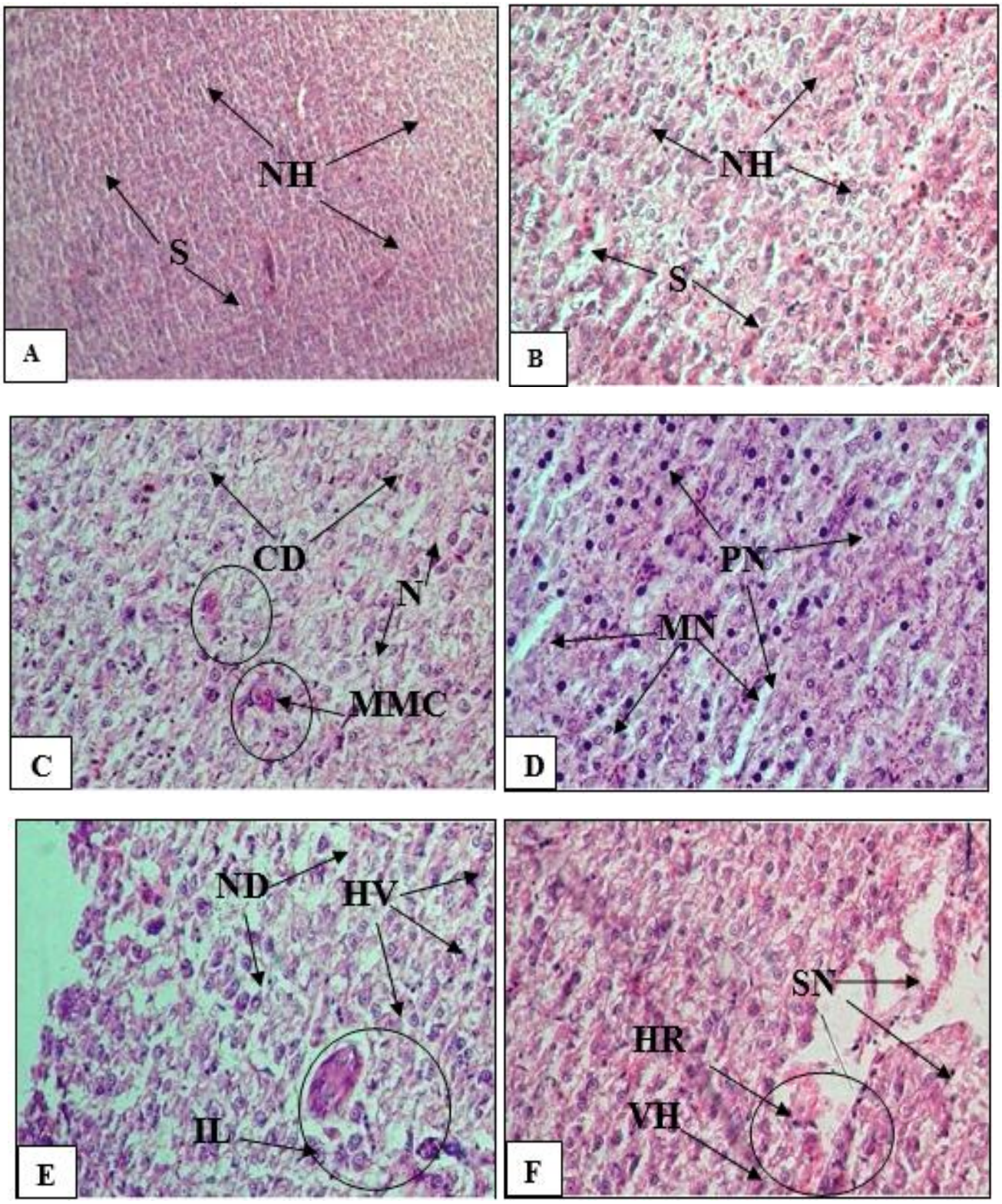

Fig 1: Transverse sections of liver stained with H & E: (A, B) Control carp showing normal hepatocytes (NH), sinusoids (S) at 100X and 400X, respectively. (C, D, E, F) Market carp showing various alterations such as CD (cytoplasmic degeneration), N (necrosis) and few MMC (melano-macrophage centres), PN (pyknotic nuclei), MN (mild necrosis), ND (nuclear degeneration), HV (hyper-vacuolization) and IL (infiltration of leukocytes), SN (severe necrosis), HR (hemorrhage) and VH (vacuolization of hepatocytes) at 400X magnification

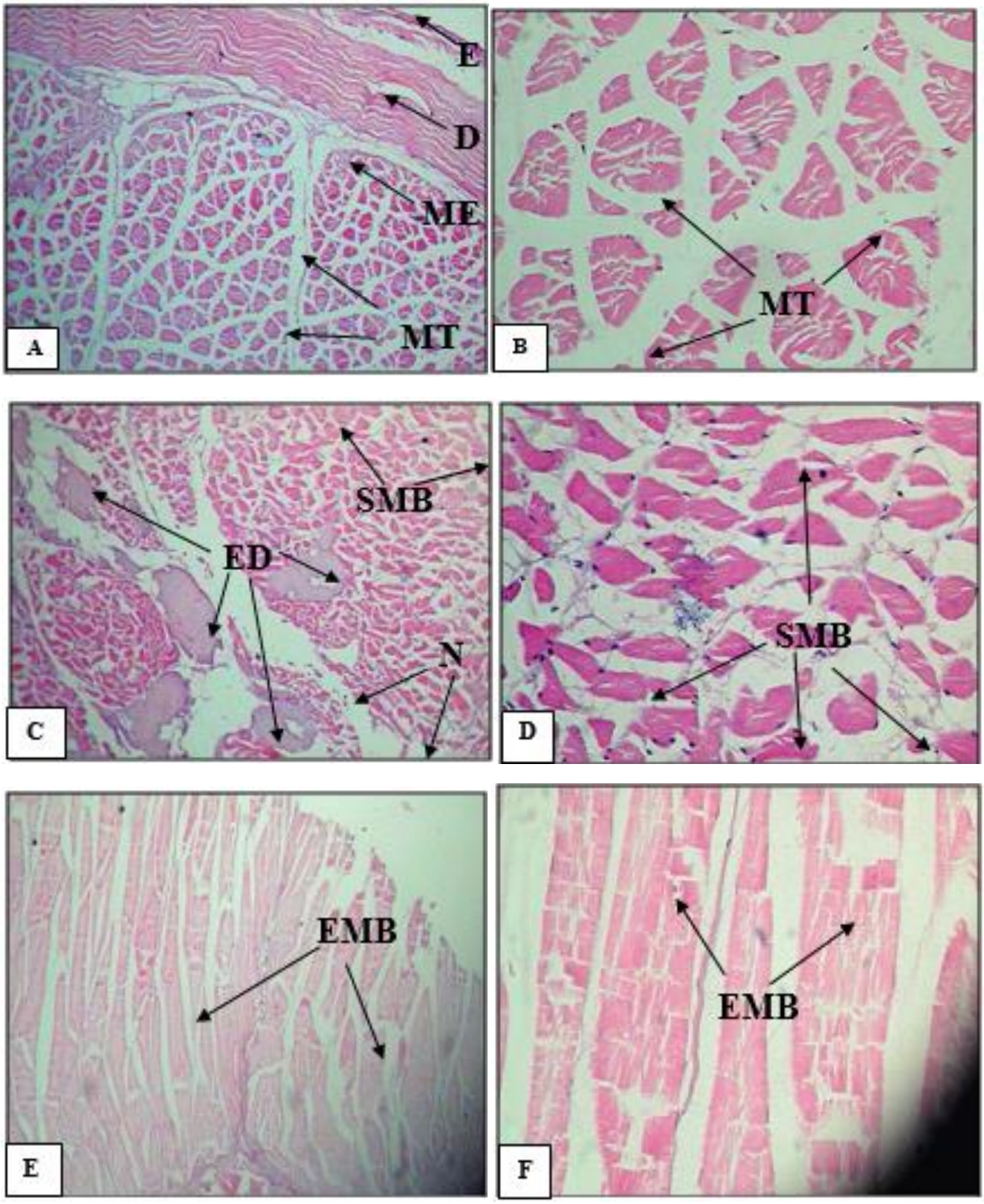

Fig 2: Vertical sections of muscle stained with H&E: (A, B) Control carp showing E (epidermis), D (dermis), ME (myoepithelium) and MT (myotomes) at 100X and 400X, respectively. (C, D) Market carp showing various alterations such as ED (edema), SMB (shortening of muscle bundles) and N (necrosis) at 100X and 400X magnification, respectively. (E, F) Market carp showing EMB (elongation of muscle bundles) at 100X and 400X magnification, respectively

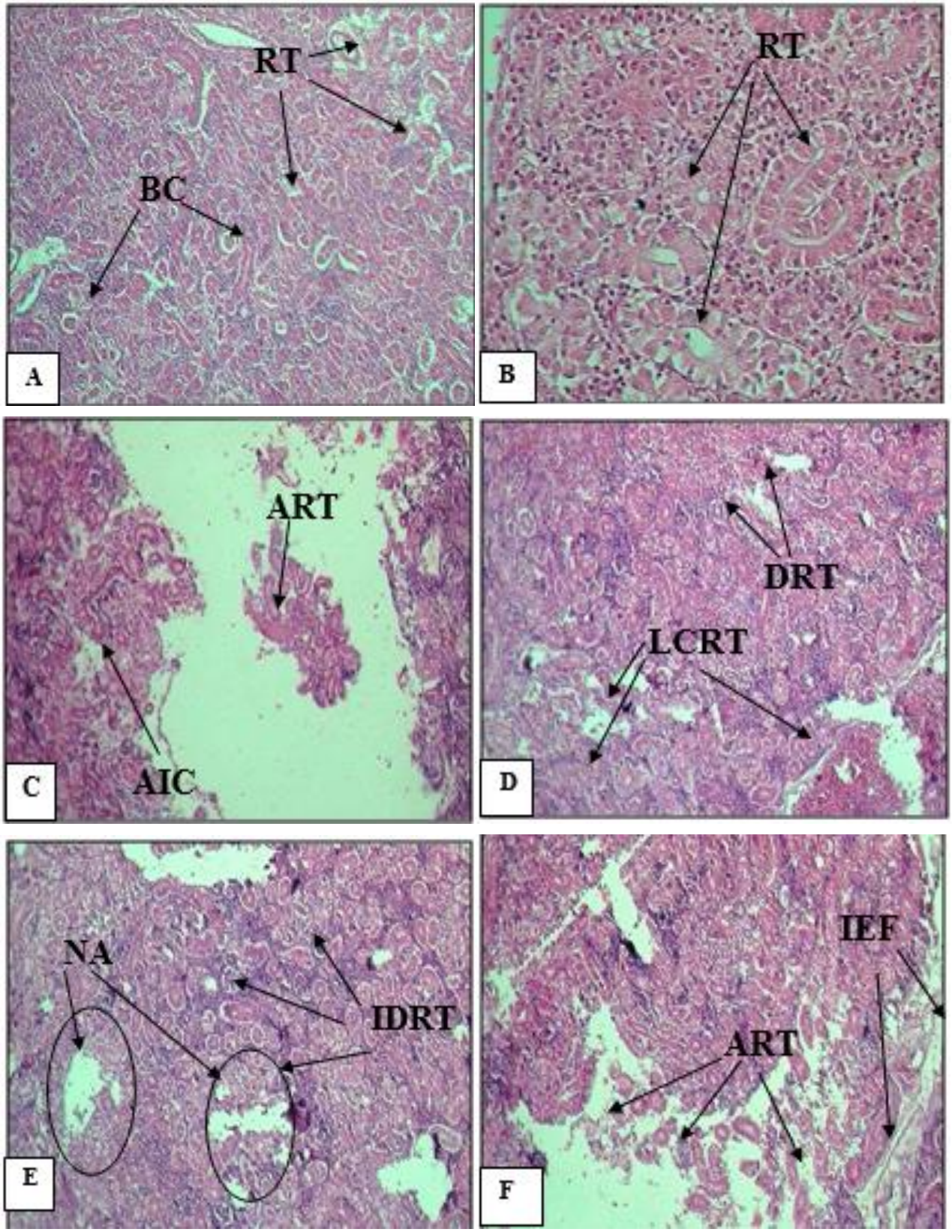

Fig 3: Transverse sections of kidney stained with H&E: (A, B) Control carp showing BC (bowman's capsule) and RT (renal tubules) at 100X and 400X, respectively. (C, D, E, F) Market carp showing various alterations such as ART (atrophy of renal tubules), AIC (aggregation of inflammatory cells), LCRT (loss of cellular integrity of renal tubules), DRT (degeneration of renal tubules), IDRT (irregular diameters of renal tubules), fewer NA (necrotic areas) and IEF (infiltration of edematous fluid between renal tubules) at 100X magnification

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