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Cytopathological changes of Selected Tissues in Asian Sea Bass, *Latescalcarifer* (Bloch) Exposed to Mercury

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Abstract
Heavy metal concentrations of mercury and the associated structural deformities in the gills, muscles, intestine, brainand liverof Asian sea bass, *Latescalcarifer* were observed for sublethal toxicity studies.Cytopathological alterations in gill tissuesdisplayed oedema of epithelial cells, ballooning dilation, partial epithelial lifting, and damaged pillar cells; the muscle displayed shortening of muscle bundles, oedema and necrosis, atrophy of muscle bundles, and vacuolar degeneration in muscle bundles;the intestine showed deformities in degeneration in cup villi, atrophy of muscularis layer, inflammation in mucosa and submucosa, and degeneration in submucosa, serosa, and tunica serosa. In the brain,the pathology was observed as necrosis of the brain cells, degeneration of nerve cells, atrophy, and dissolution of nissel bodies. The liver tissue showed degenerative hepatocytes and nuclear hypertrophy. The present study demonstrated that all the treated body tissues exhibited significant damage with response; among the body organs, the liver and brain are important target organs for mercury toxicity in *L. calcarifer*, which suggest that biomarkers are useful in assessing the model organism for toxicity studies.
Keywords:*Latescalcarifer*, mercury, cytopathology

Introduction

Mercury has no known metabolic functions in human beings and therefore even low concentrations in the body may be measured to be potentially harmful. Mercury in fish and seafood occurs mainly as methyl mercury and partly as inorganic mercury bound to organic molecules. Mercury compounds exert their action by altering the membrane structure, and thus seriously affect the permeability character of cell types. The inability of mercury - exposed fish to maintain its ionic balance could be attributed either to a decreased uptake of ions via gills or to an increased loss of ions via gills or kidney.Histopathology refers to the microscopic examination of tissues in order to study the manifestations of disease or damage. Toxicological histopathology gives useful data regarding changes induced by chemicals in pesticides at the tissue and cellular levels. All tissues and organs in the body of

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an animal are considered potential targets for the toxic effects of any chemical compound, for example pesticides. A histopathological assessment clarifies the nature of tissue alteration and the extent of damage indicating the toxic nature of the compound. The advantages of using cytopathological biomarkers in environmental monitoring are manifold. Biomarkers allow examining specific target organs, such as gills, kidney and liver, responsible for vital functions, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish [1, 2 & 3]. Furthermore, the alterations found in these organs are generally easier to identify than functional ones and they serve as warning signs of damage to animal health [4].

Changes in fish gill reflect the most commonly recognized responses to environmental pollutants [5]. Also, gills are the first target of waterborne pollutants because they are the main place for mercury uptake; they also have a constant and direct contact with the outdoor environment. Monitorisation of histological changes in fish liver is a highly sensitive and accurate way to assess the effects of xenobiotic compounds in field and experimental studies. Numerous types of gill damage have been documented in fish exposed to toxicants or in populations sampled from polluted environments [6,7&8]. Most of the gill histo-pathological changes are largely non-specific as deep-rooted by the occurrence of similar alterations under a wide range of toxicant-exposure conditions [9].The important functions of fish gills are respiration, acid-base balance, excretion, and osmoregulation and they are one of the most important organs for the uptake of inorganic mercury in fish [10] which also become efficient indicators of water quality [11 & 12]. Liver is allied with detoxification and biotransformation due to its position, function and blood and it is one of the vulnerable organsdamaged by a diversity of toxicants [13]. Heavy metal or pesticide exposure results in degenerative changes in the muscle tissue [14& 15].Stomach and intestine are important organs of digestion and absorption exposed to heavy metal pollutants. Histological alterations have been reported in the intestine of fish as a result of exposure to different toxicants [16, 17, 18&19]. Stomach of teleosts presents a variety of shapes. An acidic gastric juice is secreted by the mucosa of the stomach in carnivore's fish. Thus, stomach is an important organ in the digestive system of teleost fishes. On the contrary, the intestine is a complex multifunctional organ. In addition to digesting and absorbing feedstuffs, the intestine is critical for water and electrolyte balance, endocrine regulation of digestion and metabolism, and immunity, the cytopathological study of the stomach helps in assessing the health status as well as metabolism of fish. In this paper we report the cytopathological examination of gill, muscle,intestine, brain and liver tissues of Asian seabass, *Latescalcarifer.*

Materials and Methods

Experimental animal collection and maintenance

The experimental animal *L.calcarifer* were collected from Rajiv Gandhi Centre for Aquaculture (Tamil Nadu, India) and acclimated to laboratory conditions for 15 days. During this period, fishes were maintained in 100-L capacity aquaria with water and equipped with filter and oxygenation systems. During acclimation period, salinity (15$\pm$2 ‰), density (1.021–1.031 g/cm3), temperature (25– 26˚C) and nitrite and nitrate

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concentrations were measured and kept constant (dissolved oxygen 6.5±0.7 mg/L; hardness 100 mg $CaCO_3$/L and the absence of heavy metals). For the experimental duration , the animals were maintained under a natural light/dark cycle and fed every second day with commercial fish food. They were starved for 24 h before and during the experiment.

Chemicals used

For preparation of stock solution, 1.368 g of Mercuric Chloride ($HgCl_2$) (Merck) was dissolved in l litre of double-distilled water and used as stock solution. It was stored in a clean standard flask at room temperature in the laboratory.

Experimental procedure

Test concentration

Fish were exposed to nominal 0.127 and 0.254 ppm of mercury. Doses were theoretically sublethal, 10% and 20%, respectively, of the maximum acceptable toxicant concentration (MATC), which was 1.27 ppm. The MATC was represented as no observed effect concentration (NOEC) < MATC< LOEC (lowest observed effect concentration). The test concentration was estimated using the application factor (AF) concept, by dividing the limits (NOEC and LOEC) of the MATC by the 96h LC50 (AF= MATC/LC50 = (NOEC–LOEC)/LC50).

System design

A recirculation closed system was set up according toMuthuvan[20] . The experiment was carried out in 300 L glass aquarium (120 - 60 -50 cm), in which one compartment (50 - 50 - 40 cm) was partitioned by a plastic gauze (mesh size 1.5 mm) to contain a biofilter. Each aquarium was filled with 300 L of natural sea water (salinity of 27 ±2 ppt), which was pumped continuously over a biofilter column at a rate of 4 l/min. The water was continuously aerated throughout the experiment.

Test procedure

After 2 weeks of acclimatisation in a holding tank, ten healthy fish (7.17 ± 0.27cmin length and 9.18 ± 0.47gm in weight) were transferred to each aquarium at a loading density of 0.69 g/L. Three replicates were performed for test concentration and control. Fishes were fed twice daily with chopped fresh fish at 10:00 and 14:00 h. Uneaten food was quickly removed from the system. Fishes were starved for 24 h before sampling. The experimental water (50%) was changed every 2 weeks to keep the water quality within acceptable limits according to APHA[21]; water quality (dissolved oxygen, temperature, pH and salinity) was measured everyday and water chemistry (ammonia nitrogen, nitrite nitrogen, nitrate nitrogen) was measured twice weekly. All chemical parameters were determined following the techniques of APHA[21]using analytical grade reagents. The actual concentration of copper was measured weekly before and after its addition to maintain concentrations at the designed level. Water characteristics and the actual copper concentrations are shown inTable 1. Mortality and behaviour were observed everyday for each concentration. Two fishes from each aquarium were sampled at 0, 7 and 28 days post-exposure.

Parameters	Range	Mean+S.D
Dissolved oxygen (mg/l)	6.5–7.5	6.97±0.15

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Temperature (ºC)	25.9–28.7	27.1±1.25
Salinity (%)	26.4–32.2	29.0±1.87
pH	6.51–8.74	7.50±0.91
Ammonia nitrogen (mg/l)	0.02–0.76	0.47±0.31
Nitrite nitrogen (mg/l)	0.03–0. 73	0.64±0.51
Nitrate nitrogen (mg/l)	0.59–0.89	0.71±0.12
Actual mercury concentration (ng/l^{-1})	4.4 -5.2	0.017±0.02

Cytological analysis

The gills, muscle,intestine, liver and brain were fixed in 10% buffered formalin for 24 h, dehydrated through a graded ethanol series and embedded in paraffin. Tissue sections (5 mm thick) werestained with haematoxylin–eosin. The thin sections of the tissues were stained by haematoxylin and eosin for observation by the Nikon bright field transmission microscope with Koehler illumination and automatic exposure unit was used.

Results

Cytology of Gills

Cytological study of the gills shows a distinctive structural association of the lamellae in the untreated fish (Plate 1 A). There are four gill arches on each side of the buccal cavity and each arch is composed of numerous gill filaments. The primary gill lamellae are flat leaf - like structures with a central rod-like supporting axis and a row of secondary gill lamellae on each side of it. The secondary gill lamellae (SGL) were equally spaced along the columnar structures with intact cellular layer attached at their bases with the primary gill lamellae (PGL) and free at their distal ends. Secondary gill lamellae were composed cells, which were contractile and separated the capillary channels. The normal secondary lamellar epithelium was simple, consisting of a thin single or double sheet of epithelial cells, blood vessels and a row of pilaster cells. The region between the two adjacent secondary gill lamellae is known as interlamellar region. The lamellae are lined by a squamous epithelium composed by pavement and no differentiated cells. One to two erythrocytes were frequently observed within each capillary lumen. Chloride cells are seen as large epithelial cells with light cytoplasm, usually present at the base of lamellae. At the base of the lamellae mucous cells were present in the filamentalepithelium and they lacked the light cytoplasm.

Cytopathology of Gills

Lower concentration of mercury treatment at 7 days resulted in numerous forms of histopathological alterations in the gill filaments including lifting of lamellar epithelium (LLP) and hyperplasia (HP) in the distal region (Plate 1 B). Changes were observed as

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proliferation of filamentary epithelium (PFL), hyperplasia of the epithelial cells (HPER), and epithelial lifting (EL) in primary lamellae at lower concentration of mercury treatment in 28 days (Plate 1C). The separation of secondary gill lamellae (SSGL),and the separation of epithelial cells from pillar cells (SEPC) were noticed. The fusion was of the highest order and no distinction could be made among pillar cells, epithelial cells rupture, vaculations, blood cells and mucus cells in such region were observed in higher concentration at 7 days of mercury exposure (Plate 1D). After 28 days photomicrograph of gill treated with higher concentration of mercury showed cytoarchitectural distortion of the lamella with overlapping of the primary and secondary lamella. Considerable mucous and granulated eosinophilic cells were witnessed in their cytoplasm. Extensive vacuolisation was noticed with prominent disruption of the epithelium and the blood cells accumulated in this spherical structure. The infused secondary lamellae were thinner compared to their controls. The epithelial cells were seen between the fused secondary lamellae. Fusion of the Boundary of the secondary lamellae (FSGL) increased with exposure periods (Plate 2E). Other histopathological changes were cellular hypertrophy or hyperplasia (HP) in the epithelial layer of primary filaments and fusion of secondary lamellae. Other observations during the experimental period include epithelial rupture (ER) interstitial oedema and blood congestion in the vascular axis of primary filaments. In addition, a few telangiectasis were also observed at gill lamellae (Plate 2E). Oedema of epithelial cells, ballooning dilation, partial epithelial lifting, damaged pillar cells, enlargement of primary lamellar epithelium, aneurism of secondary lamellae, congestion of blood spaces, infiltration of the secondary lamellae (SSGL) cellular hyperplasia, mucin filled space, damaged gill rackers, fusion of secondary lamellae, infiltration of erythrocytes, degeneration of lamellar epithelium (DGL) breakage of gill rackers, necrosis of lamellae, blood congestion and hyperplasia of epithelium were also detected (Plate 1 F). The main response of gill epithelium was reduction in permeability.

Cytology of Muscle

Photomicrograph of the muscle (Plate 2A) depicted the presence of normal myotomes with equally spaced muscle bundles. Muscles are composed of segmental myomeres. Each myomere is regarded as apparent muscle and its fibres are parallel to the long axis of the body. This muscular layer is covered with skin which is formed of three layers (epidermis, dermis and hypodermis). Also, the skin is covered with an epithelial layer. The control muscle bundles were intact with signs of high metabolic activity. The skeletal muscles of fish are highly active helping in their navigation through water. Muscle bundle, muscle fibre, nuclei and endomysium are distinct. Histological study of muscle tissues of the control sea bass showed various muscle layers i.e. epidermis, dermis, myo-epithelium and normal myotomes with equally spaced muscle bundles which indicated the fish to be in unstressed conditions (Plate 2 A).

Cytopathology of Muscle

In contrast, muscle tissue of experimental sea bass at 7 days exhibited lower concentrations of mercury exposure with prominent changes like disintegrated myofibrils (DMF), and intermyofibrillar space (IMS) (Plate 2B). Granular aggregates (GA), Myofibrils of endomysium detachment (MED), and severe intramuscular oedema (SIO) were also

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observed in lower concentrations at 28 days (Plate 2C &D). The muscle of fish exposed to higher concentrations of mercury at 7 days showed detachment of muscle fibre (DMF), vacuolar degeneration in muscle bundles , spitting of muscle fibre, focal area of necrosis shortening of muscle bundles (SMB), necrosis of muscle bundles, thickening of muscle bundles (TMB) and a number of other histological changes (Plate 2 E). Exposure to sublethal concentrations of mercury at higher concentration in 28 days marked thickening and separation of muscle bundles, haemolysis, necrosis, lesions with reduced compactness and pronounced intramuscular oedema with minor dystrophic changes. Splitting of muscle fibres and vacuolar degeneration in muscle bundles were considered to be significant histopathological changes (Plate 2F).

Cytology of Intestine

The histological structure of proximal and distal intestine of sea bass showed that the basic organisation of intestinal wall was similar to that in other teleost and vertebrates formed by four layers of mucosa, sub-mucosa, muscularis and serosa. The mucosal surface forms numerous highly branched elongated, compact, deep finger like folds called villi in proximal portion and few short villi in distal portion of intestine lined by simple columnar epithelium. The cross section of the intestine in the control fish shows a collection of absorptive cells responsible for transporting important substrates from the intestine into the blood stream. Mucosa, submucosa, inner circular muscle, eosinophilic cells and the villi are well developed and highly vascularised (Plate 3 A). This epithelium consists of absorptive cells and goblet cells. The goblet cells appear like swollen, ovoid and flask shaped scattered among the absorptive cells.

Cytopathology of Intestine

When exposed to mercury in lower concentrations at 7 and 28 days the intestinal cells of sea bass undergo a number of histological changes ranging from shortening of villi, swelling of villi (SV), haemorrhage in the submucosa, inner circular muscle necrosis, rupture of basement membrane (RBM), degeneration in cup villi, atrophy of muscularis layer, inflammation in mucosa and submucosa, degeneration in submucosa, reduction of basement membrane thickness (RBMT), degeneration in serosa, degeneration of tunica serosa, damage outer circular layer, damage longitudinal muscle layer (DLML), degeneration of the connective tissue, leucocytes infiltration and abnormal lumen (AL) (Plate 3 B &C). The pathological findings in the intestine of *L.calcarifer* at higher concentrations in 7 and 28 days included atrophy in the muscularis, severe degenerative and necrotic changes in the intestinal mucosa and submucosa with necrotised cells aggregated in the intestinal lumen. Swollen villi (SV) and degenerated epithelium (DEL) were observed at higher concentrations. Haemorrhage in the submucosa and aggregations of inflammatory cells in the mucosa and submucosa with muscle fibers were seen. Oedema between them and atrophy in the submucosawas observed (Plate 3 D & E). Damage to the intestinal tissue, particularly to enterocytes and villi structures, was detected histologically. In the intestine wide spread epithelial necrosis occurred. The serosa layer enlarged and the circular muscle layer also indicated the tendency of increasing thickness. There was degeneration of intestinal folds. Due to the shrinkage of villi, the lumen of the intestine was widened (LL).

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The other cytopathological changes observed in the intestine were fibrosis associated with hyperplasia and metaplasia. The dilation of blood vessels, vacuolation of submucous cells and proliferative changes leading to the degeneration of various layers of the intestine were evident. Inflammation and fibrosis associated with hyperplasia and metaplasia were observed (Plate 3F).

Cytology of Brain

The architecture of brain in the control fish showed clear neural cells with distinct nuclei. Five major regions are distinguished in the brain of fishes. They are telencephalon, diencephalon, mesencephalon, metencephalon and mylencephalon. In fishes, the roof of the telencephalon is covered with the membranous tissue and lateral ventricles do not exist. The diencephalon is the region that contains the third ventricle and is composed of the epithalamus, thalamus and hypothalamus. The mesencephalon contains the centre of the visual sense, as well as the integration centre between this sense and the other senses of locomotion. Metencephalon occupies the interior portion of the dorsal wall of the fourth ventricle and is composed of a cortex and medulla. The metencephalon is the integration centre between the auditory sense and the sense of the lateral line. The main part of the mylencephalon, the medulla oblongata, is shaped like the spinal cord opened on its dorsal side, Pyramidal cells and Neuronal cells was observed (Plate 4A).

Cytopathology of Brain

Brain revealed generalised congestion and dilation of meningeal vessels along with infiltration of mononuclear cells. The major histological changes in the mercury treated brain of the sea bass at 7 and 28 days of lower concentrations were observed in neuronal cell degeneration (NCD) of granular and molecular layers. Vacuolisation and necrosis, Enlarged pyramid cells (EPC), Karyyorrhexis (K) and Pycnotic nuclei (PN) of the brain cells were observed (Plate 4 B &C). Other pathological changes observed in the brain of exposed fish in higher concentrationsat 7 and 28 days include neural cell degeneration (NCD), vacularisation (V), atrophy, dissolution of nissel bodies, swelling of the axon, and cellular damage in the interior and posterior regions (Plate 4 D & E). The major histological changes observed include atrophy, necrosis and dissolution of nissel bodies, swelling of the axon and vacuolisation of the myelin sheath of the nerve fibres, necrotic single cell (NSC) and neuronal degeneration (ND). The histological alterations in the brain of *L. calacrifer* exposed after 28 days showed dark-stained degenerating neurons and vacuolar changes with empty spaces. (Plate 4F)

Cytology of Liver

In the present study, the liver of control fish showed typical compact histo-architecture which was characterised by normal hepatocytes containing granular cytoplasm and nuclei and presence of sinusoids (Plate 5A). The structure of the normal liver of the fish consists of a continuous mass of large hexagonal cells. The hepatocytes are large in size with homogenous granular cytoplasm and centrally located distinct nuclei. Each cord is separated by the thick wall of the peripheral cells The control liver showed intact hepatic cells closely packed carrying large nuclei. The tissue is highly vascularised with indications of high secretary activity, hepatocytes, granular cytoplasm and centrally placed round nuclei.

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Hepatocytes are polygonal cells with a central spherical nucleus and a densely stained nucleolus. The present study also demonstrates that the liver of control fish exhibits a normal architecture and there are no pathological abnormalities. The hepatocytes present a homogenous cytoplasm and a large central or subcentral spherical nucleus (Plate 5A).

Cytopathology of Liver

The Cytopathological appearance of the liver exposed to mercury at lower concentrations in 7 and 28 daysshowed important alterations comprising degeneration of hepatocytes (DHC), swelling of hepatocytes (SHP), vauole of cytoplasm (VC), blood congestion in the central veins, as well as the diffusion of melanomacrophages in the parenchymal tissues, damaged epithelium (DE) (Plate 5B). It revealed that the increase in duration at lower concentrations causes vaculation of cytoplasm (VC) cytoplasmic degeneration (CD), damage of nuclei, and bile stagnation in addition to congestion in the blood sinusoids (Plate 5C & D) The severity of histopathological changes increased with increase in concentrations and exposure period revealed various alterations such as, cellular necrosis (CN), hepatocytes with irregular shaped nucleus (HISN) severe cell death, melano-macrophage centres, presence of pyknotic nuclei, mild necrosis, nuclear degeneration and hyper-vacuolisation. Infiltration of leukocytes in sinusoids was also noticed at 7 days (Plate 6E). Disruption of normal hepatocytes in addition to hemorrhage, swelling of hepatocytes (SHC), rupture of blood vessels ,binucleate (BN) and nuclear pyknosis (NP) was found to be the well-marked changes in histology of the liver (Plate 5F).

Discussion

Cytopathology is the microscopic study of a diseased or a damaged tissue; it is an important tool of anatomical pathology since accurate diagnosis of diseases usually requires cytopathological examination of samples. Biochemical studies may give an idea of the pathological state of the animal, yet a clear picture of cytoarchitectural changes produced during chemical intoxication can be traced clearly only by cytopathological studies. These studies help assess the extent of pollution in the ecosystem caused by pollutants such as pesticides, and offer an exceptional opportunity to detect the effect of pollutants in various organs and organ systems of any organisms.

The gills are important organs for respiration, osmoregulation, acid-base balance and nitrogenous waste excretion [22]. Gills are efficient tools for biomonitoringpotential impacts because of their contactwith water and high permeability [23]. The damage caused to gills in Spiny lobater, *P. homarus* and Asian sea bass, *L.calcarifer*could be a direct result of the heavy metal in copper whichenteredin water [24& 25]. The gills are among the most vulnerable organs of the teleostsbecause of their extrinsic location and cherished contact with water. Therefore, they are accountable to damage by irritant materials dissolved or suspended in water [26]. It is assumed that during the process of gill uptake, metals are adsorbed onto sites in cell walls and cell membranes [27]. Karlsson et al., [28] mentioned that, the increase in cellular layers of lamellar epithelium may be due to an increase in the number of mitotic divisions of the lamellar epithelium. Kantham et al., [29] suggested that the gill hyperplasia may increase epithelial thickness to retard into the blood stream. Cell proliferation with thickening of gill filament epithelium may lead to lamellar fusion. The fusion and hyperplasia of gill lamellae may be induced by the effect of the toxin altering

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glycoprotein in the mucus covering of cells, thus affecting the negative charge of the epithelium and favouring adhesion to the adjacent lamellae [30]. Mallatt [9] reported that excessive secretion of mucus, lifting of the gill epithelium and fusion of the gill lamellae were all protective mechanisms that reduce impacts of pesticides on the gill tissue. Oedema with lifting of lamellar epithelium could serve as a mechanism of defence, because separation of lamellar epithelium increases the distance, which waterborne pollutants must diffuse to reach the bloodstream [31].

Alterations like epithelial lifting, hyperplasia and hypertrophy of the epithelial cells, besides partial fusion of some secondary lamellae are examples of defence mechanisms, since; in general, these result in an increase in the distance between the external environment and the blood, they serve as a barrier to the entrance of contaminants. The increased distance between water and blood, impairs oxygen uptake [32]. These cytopathological changes of the gills likely resulted in hypoxia, respiratory failure problems with ionic and acid-base balance [5]. In addition, the pathological changes in the chloride cells may indicate osmoregulatory dysfunction, which is the main function of the chloride cells [33].

Histological study of muscle tissues of the control fish 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. In contrast, muscle tissues of experimental seabass exhibited prominent changes such as shortening of muscle bundles, oedema and necrosis (Fig. 2C, 2D). Elongation of muscle bundles was also observed. Although, muscle is the most edible part of fish body, it is also the tissue which is in close contact with pollutants dissolved in water [34& 35]. According to Saad et al., [36]if fish inhabiting polluted water displayed epithelial lesions in muscle tissues then that would most probably be invaded by micro organisms causing severe epidermal pathology, resulting in degeneration of muscle bundles. The results of the present study on market carp are corroborated by the findings of [37], who noted several histological variations such as destruction and vacuolation in the muscle cells of *Oreochromis*species, following exposure to chromium. Patnaik et al., [38] studied similarly, the histology of *C. carpio*exposed to sub-lethal concentrations of lead and cadmium. The authors reported marked thickening and separation of muscle bundles with intracellular oedema. Similarly, degeneration of muscle bundles along with 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 [39].

Due to the liver's function, position and blood supply, it is the organ most associated with detoxification and biotransformation processes [40]. It is also one of the organs most affected by contaminants in the water [41]. In the present study, the liver of control fish showed typical compact histo-architecture which was characterised by normal hepatocytes containing granular cytoplasm and nuclei and presence of sinusoids. Histological alterations are reported in the liver of fishes exposed to industrial pollutants [42]. Metals caneither increase or decrease hepatic enzyme activities and can lead to histopathological hepatic changes, depending on the metal type and concentration, fish species, length of exposure and other factors [43]. Hepatocytes are hence expected to be the primary targets of toxic substances, providing an excellent biomarker of aquatic pollution [44]. The monitorisation

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of histological changes in fish liver is a highly sensitive and accurate way to assess the effects of xenobiotic compounds in field and experimental studies [4].Our results are well supported by the work of Saini[45] who recorded severe histopathological lesions such as infiltration of lympho-nuclear cells, degeneration of hepatic parenchyma and deformation of hepatocytes, caused by heavy metals, in the liver of *L. rohita*caught from natural freshwaters of Punjab. Hypertrophy is characterised by an increase in the cellular size. Exposure to compounds that induce proliferation of endoplasmic reticulum membranes can be regarded as an example of hypertrophy [4]. Some studies reported that interstitial hepatocytes of Nile tilapia exposed to contaminated sediment showed hydropic swelling [46]. FigneiredoFernandes et al., [5] suggested that increases in the hepatocyte size may be due to high contents of lipids. On the other hand, Braunbech[47] reported that alterations in size and shape of the nucleus have been regarded as signs of an increased metabolic activity, but they may be of pathological origin. Vacuoles in the cytoplasm of hepatocytes can contain lipids and glycogen, that are related to the normal metabolic function of the liver [10]. Vacuolisation of hepatocytes might indicate an imbalance between the rate of synthesis of substances in the parenchymal cells and the rate of their release into the systemic circulation [48]. Pacheen et al., [49] described increased vacuolisation of hepatocytes a signal of degenerative process that suggests metabolic damage, likely related to exposure to contaminated water. Vacuole formation was considered by Mollendroff[50] as a cellular defence mechanism against substances injurious to hepatocytes this mechanism is responsible for collecting the injurious elements and preventing them from interfering with biological activities of these cells

The architecture of brain in the control fish showed clear neural cells with distinct nuclei. Brain is the controlling centre of all functions and movements in the body organisms like fish serving as a relay station. In the present study hyperplasia, oedema, necrosis and an increase in brain cells were some of the histological changes observed in the brain of the fish exposed to sub lethal concentrations of mercury toxicity. Mercury in the present study has induced pronounced pathological changes in the brain of the fish *L.calcarifer*. The cytopathological responses of the fish reveal the degree of damage to the brain of the fish, caused by this heavy metal. The extent of damage caused and the degenerative changes that occurred in the brain of the fish due to mercury were progressive over the period of exposure, suggesting that the cytopathological responses depend not only on the concentration of metals but also on the length of the fish exposure period to mercury.

Basantakumar et al., [51] observed mild vacuolar changes in the cerebrum with empty spaces after 0.35 ppm of hexachlorocyclohexane exposure, whereas at 1.73 ppm they have observed severe necrosis of neuronal cells of cerebrum and loss of Nissl substance in the brain of the Indian major carp (*Labeorohita*), exposed to hexachlorocyclohexane, the findings are similar to ours in present study in Asian sea bass. Ayyola and Ajani [52] reported mononuclear infiltration, neuronal degeneration and severe spongiosis in the brain of the fish *Clariasgariepinus*after exposure to lethal concentrations of cypermethin. There was a severe congestion, mononuclear infiltration, haemorrhage and generalisedspongiosis in the brain of the fish *Oreochromisniloticus*exposed to lethal concentrations of glyphosate [53]. The experimental fish brain showed disintegration and severe damage in the brain

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cells along with break down of neural bundles after exposure to different concentrations of mercury.

The impairment of tissues of a region in the brain by these pathological changes may lead to the curtailment of the particular function in fishes. This alters the physiological and behavioral functions of the fish. This is evidenced in the behaviour of the fish in the form of respiratory distress, loss of equilibrium and erratic swimming. Cope et al., [54]observed vascular dilation in fish brain on exposure to 2, 4-D and endosulfanrespectively. Pugazhvendan et al., [55] observed scattered arrangement of cells, severe necrosis and loss of differentiation in the brain cells in *Ophiocephaluspunctatus*exposed to malathion pesticide. Vacuolisation in brain tissues may be the result of glycolysis leading to microsomal and mitochondrial dysfunctions. In this study severe necrosis of neuronal cells in the cerebrum indicates a loss of nissl substances due to 10ppm exposure to zinc,which is in agreement with the study of [56].

Findings from histological studies of the gastrointestinal tract across the species of fish are more beneficial to researchers; more information is needed with regard to feeding and nutrition. In the present study, the wall of the intestine of *L. calacrifer*histologically comprised of four different layers, mucosa, submucosa, muscularis and serosa that were similar to other teleost fishes like *Clariusbatrachus*[57] *Salmosalar*[58] *Hypophthalmichthysnobilis*[58].Bhatanagar et al., and Mohamed [60& 61] observed irritation and destruction of the mucosa membrane of the intestine, that can hamper absorption. Epithelial degeneration, inflammatory cell infiltration in the submucosa and submucosaloedema were observed in the intestine of tilapia exposed to carbofuran[62]. The cytotopathological alterations observed in the intestine of Asian seabass revealed severe degenerative and necrotic changes in the intestinal mucosa. Oedema between submucosa and mucosa may be a result of the absorption of toxic metals. The present results are in agreement with those observed by investigators studying the effects of metals on fish intestine [63]. Kanoud et al., [64] reported that the pathological findings in the intestine of *Oreochromisniloticus*treated with cadmium included atrophy in the muscularis, degenerative and necrotic changes in the intestinal mucosa and submucosa with necrotised cells aggregated in the intestinal lumen, and edema and atrophy in the submucosa. These findings are similar to findings on*PimephalespromelasandChannapunctatus*exposed to cadmium [65] and lead [66].

Conlusion

Most studies on mercury toxicity in aquatic organisms have considered the exposure media in accordance with the most abundant form/species of mercury found in the uptake pathway. Therefore, effects of exposure to inorganic mercury via water have been neglected in the past studies, even though it can be accumulated after ingestion. Results of the present research show that the effect of mercury concentrations were tested in various tissues using the cytological techniques in fish. This indicates higher susceptibility of larvae to mercury exposure which could increase vulnerability to predation and therefore, endanger fish populations in contaminated environments. Changes in juvenile fish biomarkers were observed and were dependant on the tissue type and period of exposure. Hence this study can be used as a tool for creating awareness among the local farmers and compare the

sensitivity of various species of aquatic animals and potency of effluent using LC_{50} values and to derive safe concentration so that the use of the highly toxic heavy metal can be minimized.

Conflict of Interest : The authors declare that they have no conflict of interest

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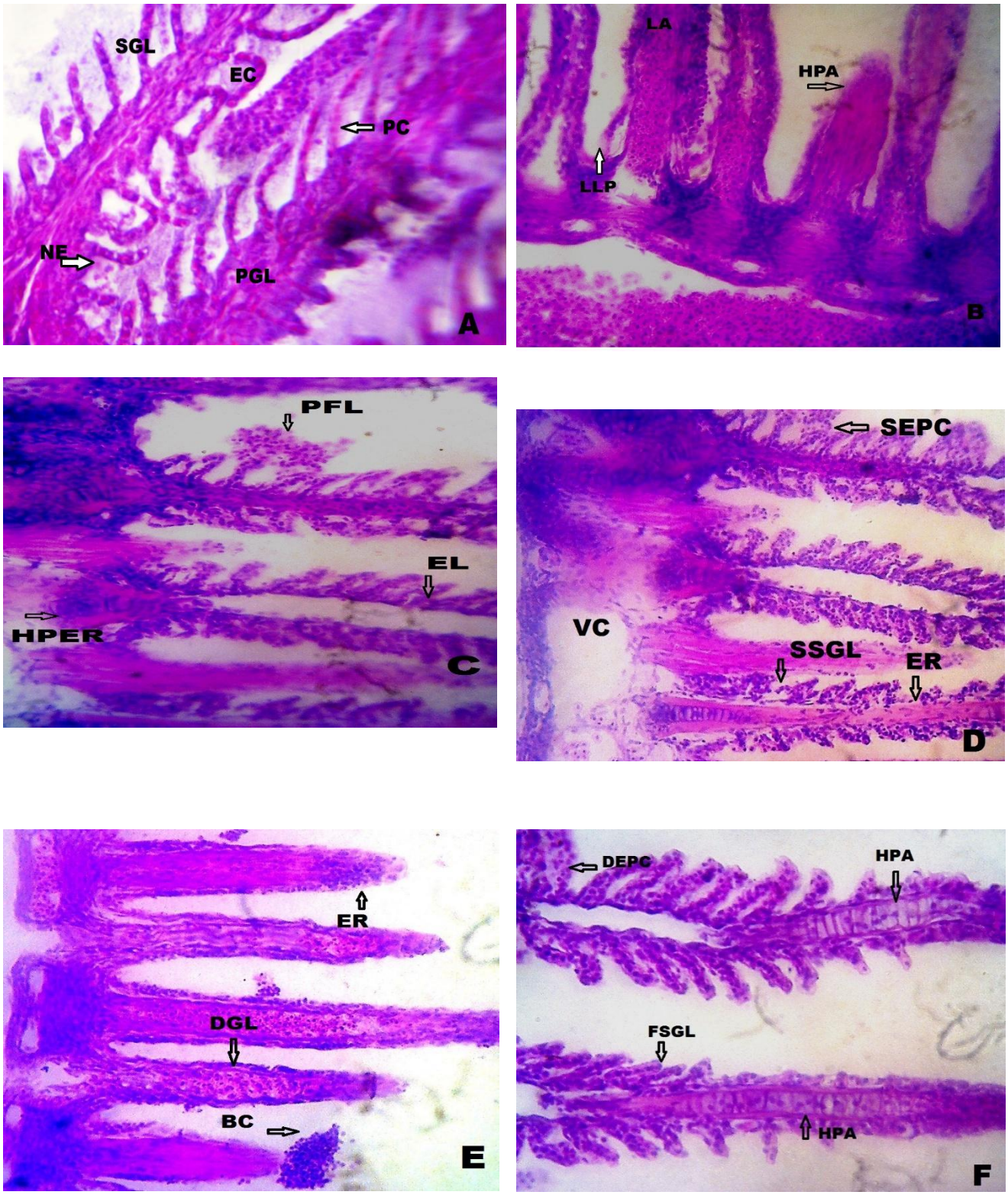
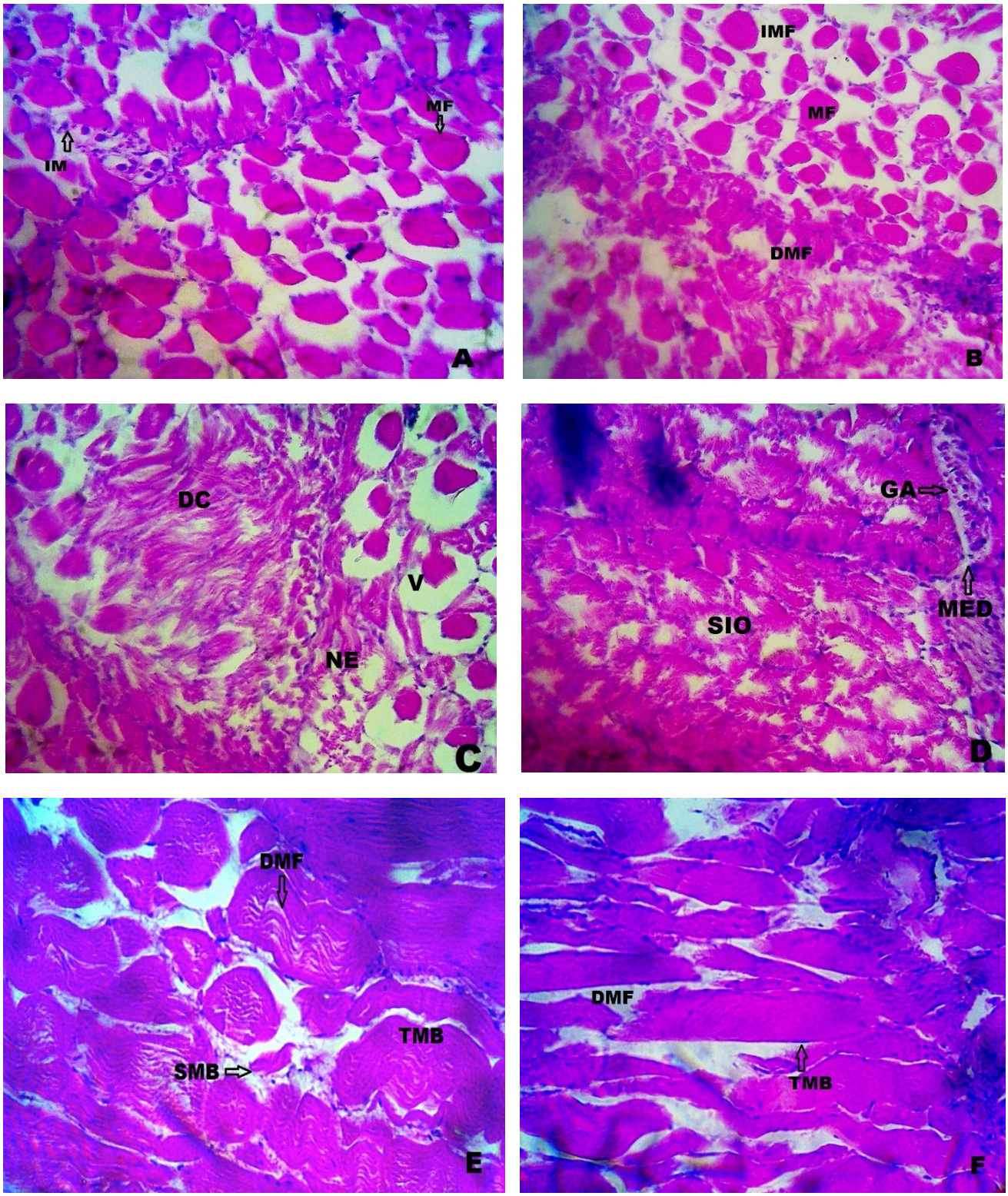
Received 16 February 2021; Accepted 08 March 2021.

PLATE : 1



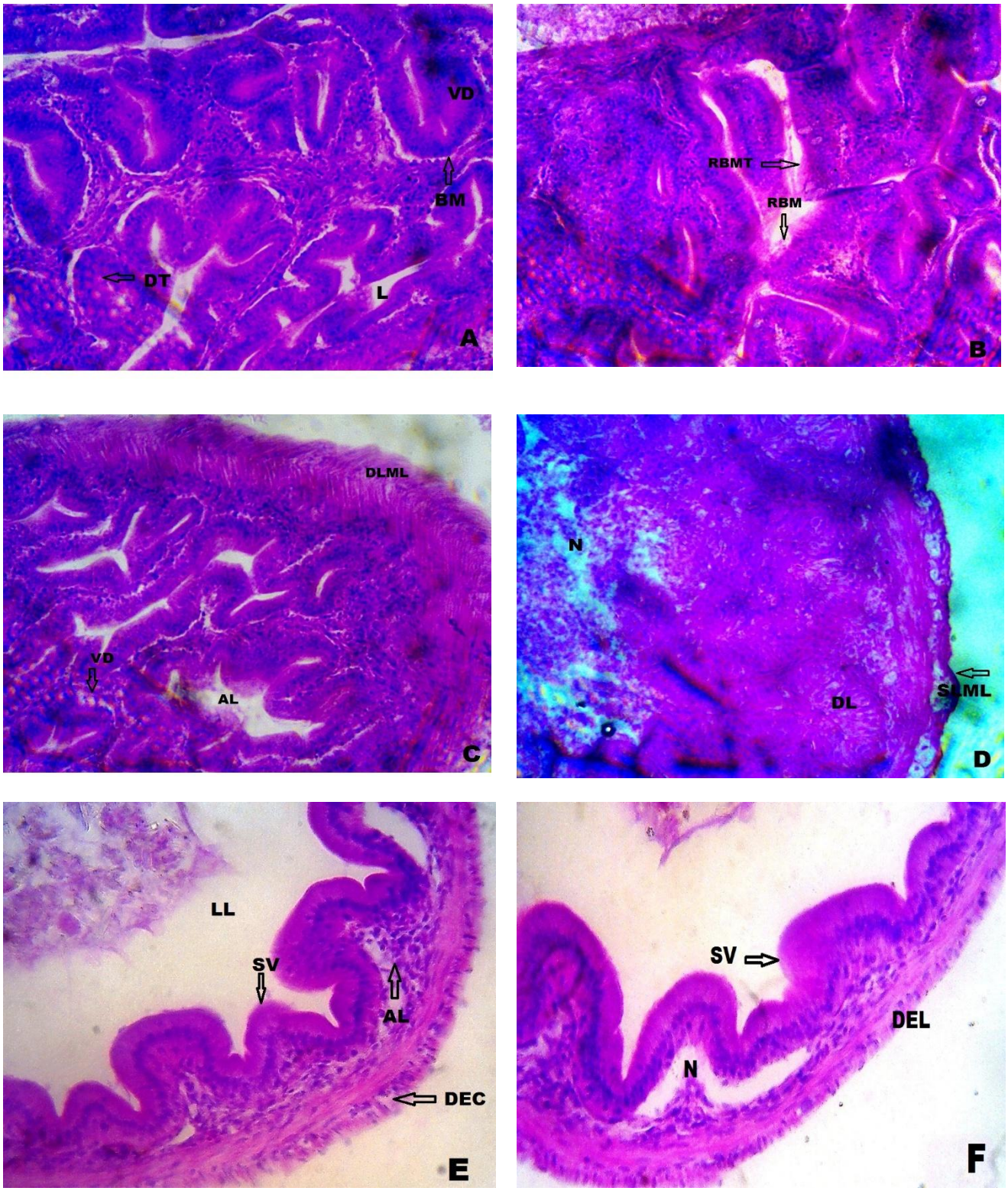
Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 1749 - 1772
Received 16 February 2021; Accepted 08 March 2021.

PLATE : 2



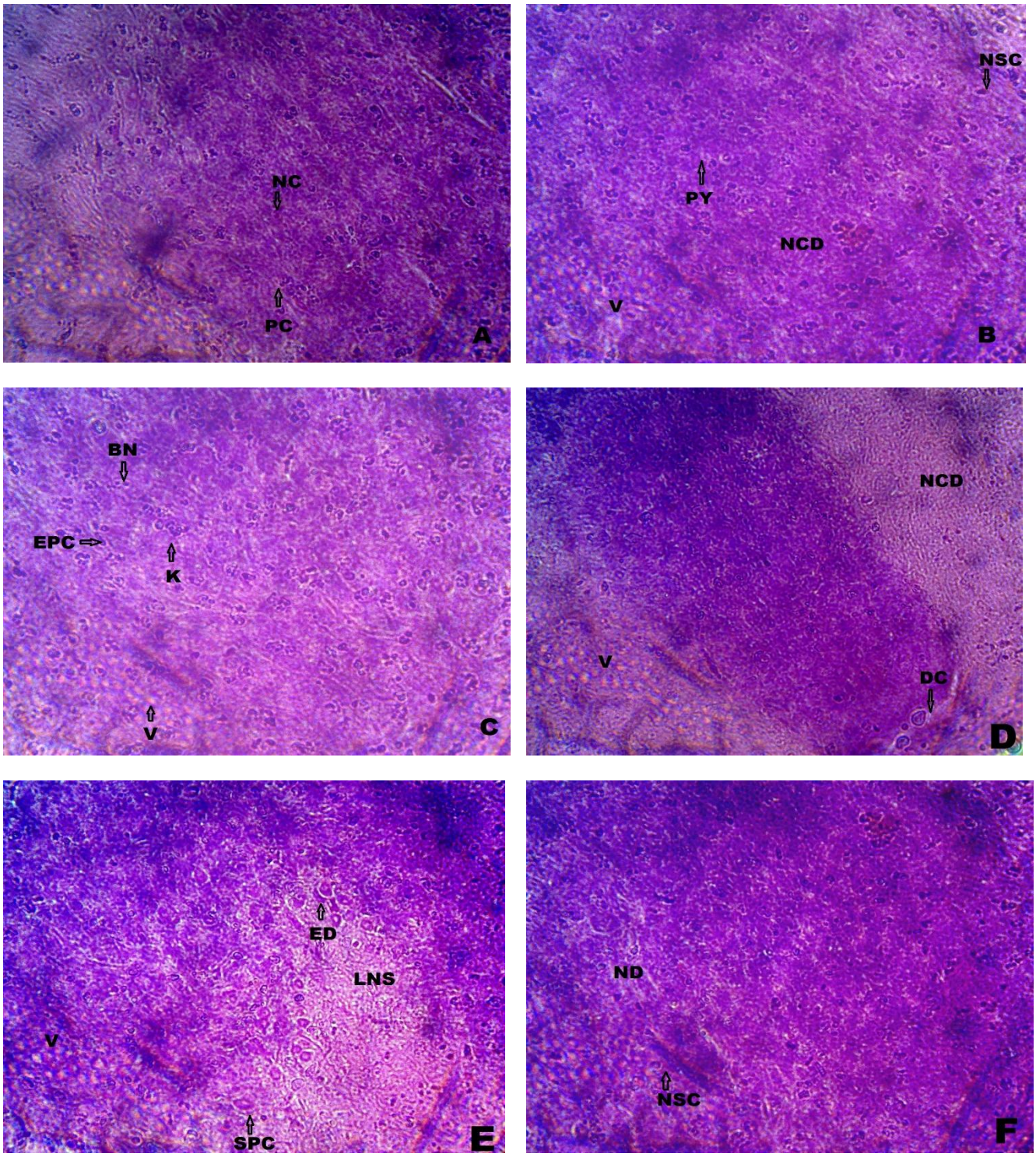
Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 1749 - 1772
Received 16 February 2021; Accepted 08 March 2021.

PLATE : 3



Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 1749 - 1772
Received 16 February 2021; Accepted 08 March 2021.

PLATE : 4



Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 1749 - 1772
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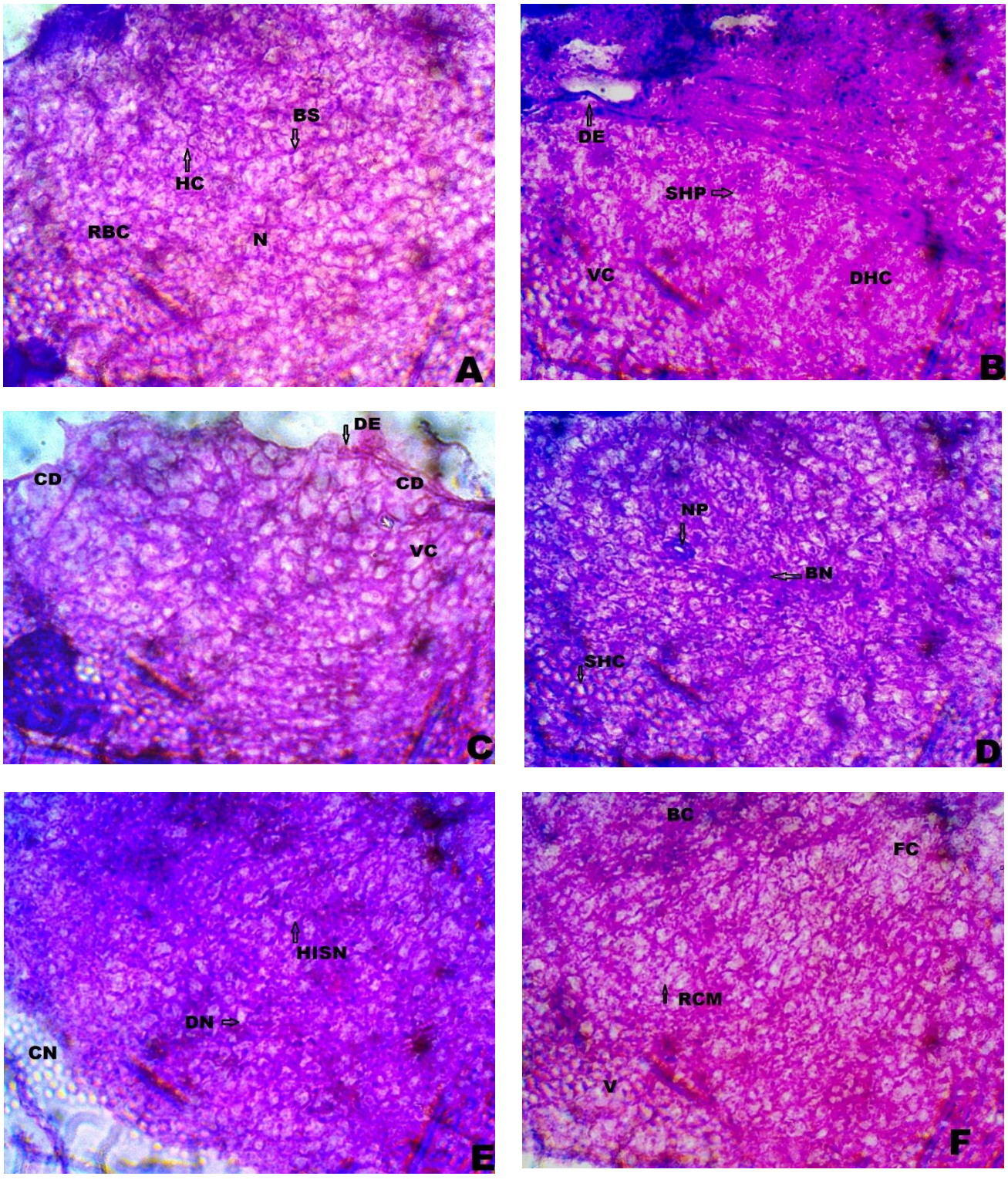
PLATE : 5



Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 1749 - 1772
Received 16 February 2021; Accepted 08 March 2021.

Plate: 1 Cytological changes of gills in *L.calcarifer*

Light micrographs of a paraffin section stained with Hematoxylin and Eosin (40x)

A- Control
B- After 7 days of exposure to 0.127 ppm concentration of mercury
C- After 28 days of exposure to 0.127 ppm concentration of mercury
D- After 7 days of exposure to 0.254 ppm concentration of mercury
E & F- After 28 days of exposure to 0.254 ppm concentration of mercury

Abbreviations used:

PGL - Primary gill lamellae
SGL - Secondary gill lamellae
PC - Pillar cells
NE - Nucleated erythrocyte
EC - Epithelial cells
HPA - Hyperplasia
LA - Lamellar aneurysm
LLP - Lifting of lamellar epithelium
PFL - Proliferation of filamentary epithelium
EL - Epithelial lifting
HPER - Hyperplasia of the epithelial cells
VC - Vaculation
SSGL - Separation of secondary gill lamellae
ER - Epithelial rupture
SEPC - Separation of epithelial cells from pillar cells
DGL - Degenerated gill lamellae
BC - Blood congestion
DEPC - Degeneration epithelial cells
FSGL - Fusion of secondary gill lamellae

Plate: 2 Cytological changes of muscle in *L.calcarifer*

Light micrographs of a paraffin section stained with Hematoxylin and Eosin (40x)

A- Control
B- After 7 days of exposure to 0.127 ppm concentration of mercury
C & D- After 28 days of exposure to 0.127 ppm concentration of mercury
E- After 7 days of exposure to 0.254 ppm concentration of mercury
F- After 28 days of exposure to 0.254 ppm concentration of mercury

Abbreviations used:

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 1749 - 1772
Received 16 February 2021; Accepted 08 March 2021.

IM - Intestitial materials
MF - Muscle fibre
IMF - Inter myofibrillar space
DMF -Disintegrated myofibrils
DC - Dystrophic changes
V - Vacuole
NE - Necrosis
GA - Grannular aggregates
SIO - Severe intramuscular oedema
MED - Myofibrils to endomysium detachment
SMB - Shortening of muscle bundle
TMB - Thickening of muscle bundle

Plate: 3 Cytological changes of intestine in *L.calcarifer*

Light micrographs of a paraffin section stained with Hematoxylin and Eosin (40x)

A- Control
B- After 7 days of exposure to 0.127 ppm concentration of mercury
C - After 28 days of exposure to 0.127 ppm concentration of mercury
D - After 7 days of exposure to 0.254 ppm concentration of mercury
E & F- After 28 days of exposure to 0.254 ppm concentration of mercury

Abbreviations used:

L - Lumen
BM - Basement membrane
DT - Digestive tubule
VD - Villi
RBMT - Reduction of basement membrane thickness
RBM - Rupture of basement membrane
DLML - Damaged longitudinal muscle layer
VO - Vacuolization
AL - Abnormal lumen
DL - Distended lumen
N - Necrosis
SLML - Swelling of longitudinal muscle layer
LL - Large lumen
SV - Swellen villi
DEC - Damaged epithelial cells
DEL - Damaged epithelial layer

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 1749 - 1772
Received 16 February 2021; Accepted 08 March 2021.

Plate: 4 Cytological changes of brain in *L.calcarifer*

Light micrographs of a paraffin section stained with Hematoxylin and Eosin (40x)

A- Control
B- After 7 days of exposure to 0.127 ppm concentration of mercury
C - After 28 days of exposure to 0.127 ppm concentration of mercury
D - After 7 days of exposure to 0.254 ppm concentration of mercury
E & F- After 28 days of exposure to 0.254 ppm concentration of mercury

Abbreviations used:

NC - Neuronal cells
PC - Pyramidal cells
PY - Pyknotic stage
NCD - Neuronal cell degeneration
V - Vacuolization
NSC - Necrotic single cells
BN - Binucleated nuclei
EPC - Enlarged pyramidal cells
K - Karyorrhexis
NCD - Neuronal cell degeneration
DC - Dystrophic changes
ED - Oedema
LNS - Loss of nissal substances
SPC - Swelling of pyramidal cells
ND - Neuronal cells damaged

Plate: 5 Cytological changes of liver in *L.calcarifer*

Light micrographs of a paraffin section stained with Hematoxylin and Eosin (40x)

A- Control
B- After 7 days of exposure to 0.127 ppm concentration of mercury
C - After 28 days of exposure to 0.127 ppm concentration of mercury
D - After 7 days of exposure to 0.254 ppm concentration of mercury
E & F- After 28 days of exposure to 0.254 ppm concentration of mercury

Abbreviations used:

BS - Blood sinus
HC - Hepatocyte
RBC - Red blood cells

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 1749 - 1772
Received 16 February 2021; Accepted 08 March 2021.

N	- Nucleus
DE	- Damaged epithelium
SHP	- Swelling of hepatocytes
VC	- Vacuoles in cytoplasm
DHC	- Degeneration of hepatocyte
CD	- Cytoplasmic degeneration
NP	- Nuclear pyknosis
BN	- Binucletae
SHC	- Swelling of hepatocyte cells
HISN	- Hepatocytes with irregular shape nucleus
CN	- Cellular necrosis
DN	- Disappearance of nucleus
BC	- Blood congestion
RCM	- Rupture of cell membrane
FC	- Fusion of cells
V	- Vacuolization