STUDY OF LEAD INDUCED HISTOPATHOLOGICAL ALTERATIONS IN CRUCIAN CARP (*CARASSIUS AURATUS*)

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Abstract : A relatively smaller member of the carp family, the gold fish was one of the earliest fish to be domesticated, and is still one of the most commonly kept aquarium fish. Most of the farmers associated with ornamental fish farming in India practice captive culture of goldfish. Like all other members of carp family gold fish too have good life span and it is also a prolific breeder. Its potential to tolerate and survive over the extreme conditions of temperature, pH, salinity, etc., makes it an excellent laboratory model to carry out various research studies. It is also known for its efficiency for being a mosquito bio-control agent apart from being just an ornamental fish. The fish is regularly used as model to study fish behavior, teratogenesis and ecotoxicity, which includes pesticide as well heavy metal toxicity. Several heavy metals find their ways in natural water bodies and affect aquatic animals adversely. Lead, in particular is highly toxic and carcinogenic to all animals including many aquatic organisms, because of its high toxicity, non-biodegradability and tendencies to undergo bio-magnification. Lead accumulates in the body of fish either through water or food. In the present investigation, adult live specimens of goldfish, *Carassius auratus* were procured from local vendor each weighing approximately 20 grams. They were first acclimatized to the laboratory conditions and then exposed to analytical grade lead acetate solutions at the concentration ten times lower than the recorded LC50 value. The fish were exposed to lead acetate for three different durations. The control set was maintained separately. Tissues were obtained for histopathological studies as autopsy samples. Sections of the tissues were stained with conventional HE stain. Histopathological observations included hepatic lesions, cytoplasmic degeneration, necrosis, nuclear degeneration etc. Shortening of muscle bundles were also evident. Irregular diameters, degeneration and atrophy of renal tubules and oedema were noticed in renal tissue. The results indicated the effect of lead toxicity nearly on all the vital organs.

***IndexTerms* - heavy metals, *Carassius auratus*, liver, kidney, muscle, histopathology.**

I. INTRODUCTION

In last few decades increase in population density, heavy industrialization and agricultural activities have resulted in more and more wastes entering in fresh water resources [1].Heavy metal pollution in water bodies has been observed as a serious concern as it is increasing steadily throughout the world each year. This is due to the release of pollutants from the various sources of industrial, agricultural and mining waste such as leaching of mineral and soil erosion as well as anthropogenic activities either directly or indirectly into the aquatic system, which has resulted into ecological imbalance of different systems[2]. Heavy metal pollution is unsafe for all living organisms, including aquatic organisms and humans. Out of all types of ecosystems, aquatic systems are more sensitive to heavy metal pollution, and the level of such heavy metals in aquatic environments increases due to anthropogenic activities [3].

Among the aquatic fauna, fishes besides being organisms with high economic value in India, they are also known to responds to environmental changes as they are explored freely among the different trophic levels in an aquatic environment, , therefore being a good bio-indicator. Moreover, fish is a good bio-accumulator as it has the optimum size for analysis and a long lifespan and is easily obtained in large quantity to be sampled for accumulated metals. Fish tends to accumulate heavy metal contaminants by many 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) [4]. Bioaccumulation is a process where chemicals invade an organism either through exposure to a contaminated medium or through consumption of food containing the chemicals [5]. Bioaccumulation in fish usually occurs when it is exposed to wide range of chemical pollutants which includes organic pollutants like germicide, insecticide, rodenticide, pesticides, etc., and inorganic pollutants like mineral acids, inorganic salts, trace elements and heavy metals. Heavy metals being ubiquitous in nature are found to be major stress related pollutant due to their property of being non-biodegradable, persistent and their durability to cause damage to the aquatic bodies [6]. Any metal or metalloid having relative atomic density more than 5 g/cm3 is considered as heavy metal [7] . Further they are classified into essential, semi-essential and potentially-toxic metals. Harmful effect can be seen by the ingestion of heavy metals even at a very low concentrations, adding to this studies reveals that essential elements also shows damaging effect when explored for longer duration [8].Amongst all the known heavy metals, Lead (Pb), is an non-essential heavy metal but potentially toxic, hence, not needed by any living organism even in a trace amount [1]. Lead being a potent pollutant; its toxicity has become a great issue of concern. Pb gets manifested by food ingestion and breathing. Pb accumulates in the muscles, bones, blood and fat [9] .Once the chemical pollutants enter the fish's body; it can damage and weaken the mechanism concerned, which leads to physiological, histopathological and biochemical alterations.

II. MATERIALS AND METHODS

2.1. Experimental Animals:

Healthy and active Adult carp *Carassius auratus* (Family: Cyprinidae), were purchased from the local vendor, Kalyan (19.2403º N, 73.1305º E), Maharashtra, India. *C.auratus* was acclimatized in the laboratory conditions as per the OECD, EPA guidelines. On the arrival in the laboratory, the fish were placed in large tanks with aerated tap water. Fish were acclimatized for 2 weeks prior to the experiment under a natural photoperiod of 12-12 h. The tap water used for fish aquaria and later during experiment had a pH value of 7±0.2 and a total hardness of 20 mg $CaCO_3$/L was renewed every day maintaining the temperature between the physiological tolerable limits of 25-30°C. Other physical and chemical parameters like dissolved oxygen, dissolved carbon dioxide, salinity were analysed using standard protocol [10].

Adult Carp (7 in each aquarium) of 20 ± 0.5 gms weight irrespective of their gender were selected for the exposure. Further, acclimatized to the laboratory conditions in 20L glass aquaria (39"X39") containing dechlorinated tap water of the quality used in the test. The fish were fed regularly with fish food pellets available commercially during acclimatization and test periods, but was stopped one day prior to exposure to the test medium for acute toxicity test only. The LC50 with 95% confidence limits for Lead were estimated for 96 h by probit analysis (Finney, 1971). The fish were then exposed to analytical grade lead acetate solutions at the concentration ten times lower ($1/10^{th}$ value =2.9mgL^{-1}) than the recorded LC50 value (29.07mgL^{-1}). The fish were exposed to lead acetate for four different durations of 7, 14, 21 and 45 days. The control set was maintained separately.

2.2. Microscopic Examination and Histopathological studies

At the end of the exposure period, adult carp were taken from each replicate tank. The gill arches of the fish were excised from both sides and were rinsed with distilled water before adding to fixative. Fish were dissected, the abdominal cavity was operated and the liver and kidney along with muscles were excised of fats and adhering tissues and quickly were fixed in the labelled bottles containing 10% formalin as a histological fixative for 24 h. The specimens were further processed as usual by the standard method of dehydration, cleared in xylene and finally embedded in paraffin wax before being sectioned using a rotary microtome (Leica RM 2125 RTS).

III. RESULTS AND DISCUSSION

3.1.Histlogy of gills:

Section of gills from the control group (figure.1) revealled normal histologicall structure under low power. The gill arches were found supporting the gill fillaments which intern hold two rows of secondary lamellae each. The secondary gill lamellae were found to be well separatted from each other showing no congestion. Efferent branchiole arteriole (EBA) and Efferent branchiole artey (EBAy) showed no blood congestion.

In 7 days (figure.2) treatment group the gills showed a few changes. Primary (PL) and secondary lamellae (SL) were found to be degenerated , while the central axis (CA) of each gill filament was found to be moderately dilated. Gill lamellae showed flask shaped ends due to epithelial lifting (EL) in 14 days (figure.3)treatment group under low power. Secondary lamellae were found to be estreamly short while the efferent granchiole artery was dilated. In 21 days (figure.4) treatment group the gill filaments (GF) were found to be elongated with completely dehydrated secondary lamellae. Inter fillamental distance was found to be reduced and the central axis was atrophied. After 45 days (figure.5) treatment the gills were completely dehydrated evidently seen in the form of broken and shrunken gill fillaments , near absence of secondary lamellae, distorted central axis and damaged gill rackers (GR).

3.2.Histlogy of Muscles:

Longitudinal section of muscles from control group (figure.6) revelled normal merphology with intact dermis (D) and dense myotome (MT). In 7 days (figure.7) treatment group it was found that the myotome was sparse while in 14 days (figure.8) treatment group they were thin, broken and elongated. Progressive distruction in the muscular morphology causing muscular atrophy (MA) was senn in both 21(figure.9) and 45 days (figure.10) treatment group as fibers were found to broken and sparse mucles (SM).

3.3.Histology of liver:

Liver tissue showed normal appearance in the histological preparation in control group (figure.11). Normal hepatocytes (HC) longitudinally cut central vein and sinusoidal spaces were seen in the tissue. After 7 days (figure.12) widder sinusoidal spaces gave columnar appearance to the hepatocytes and at certain places degenerative changes were found. After 14 days (figure.13) treatment the histological preparations showed melanomacrophage centers in certain places (MMC). In 21 days (figure.14) and 45 days (figure.15) treatment samples hepatocyte hypertrophy (HHC), pycnotic (PN) and degenerative nuclei (DN) were found.

3.4.Histology of kidney:

Kidney in its transverse section from control group (figure.16) showed normal structure. Normal appearance of Bowman's capsule (BC) and renal tubule (RT) was seen. In 7days (figure.17) treatment sample hypertrophy of renal tubule (HRT) was evident in most of the places. With 14days (figure.18) treatment loss of cellular integrity of renal tubule (LCIRT) was noticeable. With further advancement in the treatment i.e. after 21 days (figure.19) the renal tubules appeared atrophied (ART) while after 45 days(figure.20) some more degenerative changes were seen in the form of degeneration of renal tubules (DRT) and necrosis of renal tubules(NRT) in certain areas of the renal tissue.

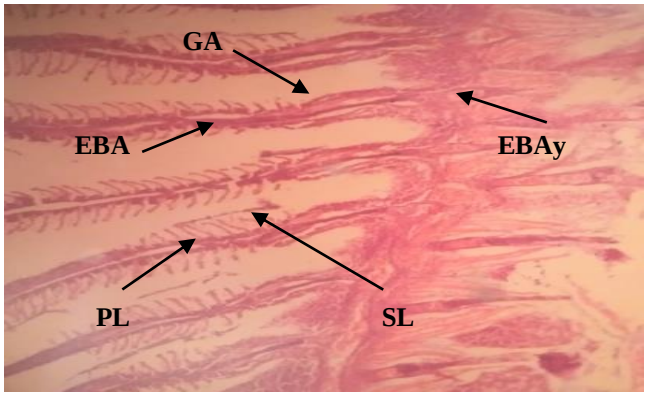

fig.1. sagittal section of gills from control group, x100.
GA- gills arches, EBA- efferent branchial arteriole
EBAy- efferent branchial artery PL- primary lamallae, SL-secondary lamallae

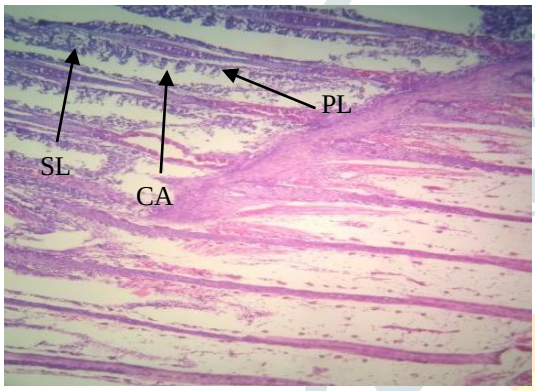

fig.2. sagittal section of gills from 7 days of exposure group, x100.
PL- primary lamellae, SL- secondary lamellae, CA- central axis

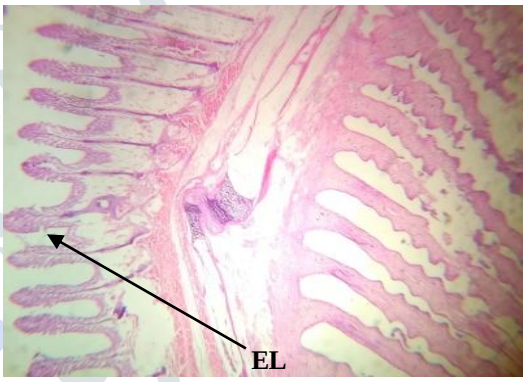

fig.3. sagittal section of gills from 14 days of exposure group, x100 .
EL- epithelium lifting

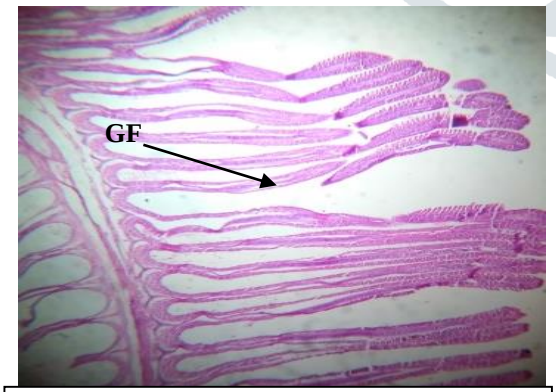

fig.4. sagittal section of gills from 21 days of exposure group, x100 .
GF- gill filament

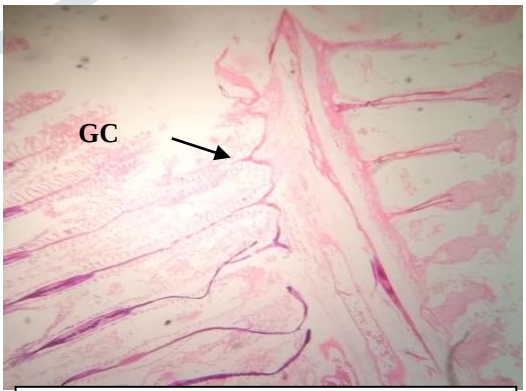

fig.5. sagittal section of gills from 45 days of exposure group, x100 .
GC- gill cracker

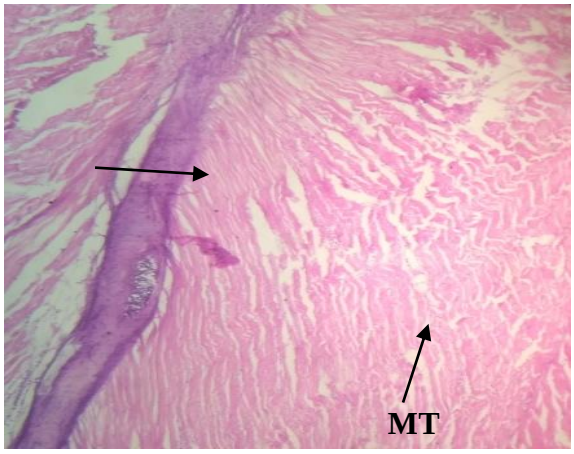

fig.6. sagittal section of muscles from control group, x100 .
D- dermis, MT- myotome

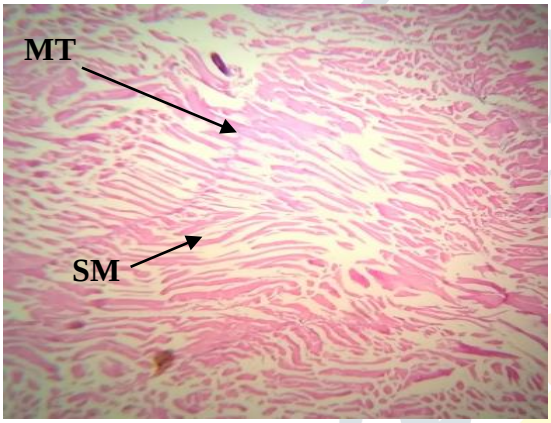

fig.7. sagittal section of muscles from 7days of exposure group, x100 .
MT- myotome, SM- sparse muscles

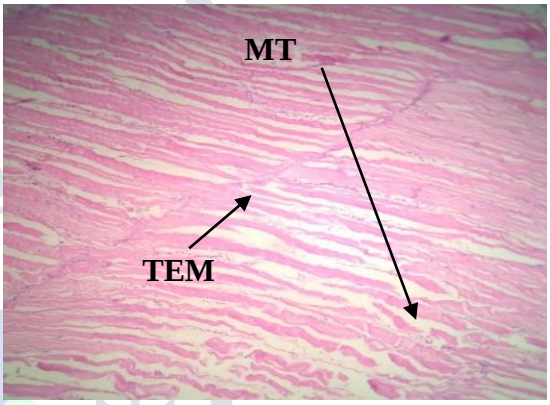

fig.8. sagittal section of muscles from 14 days of exposure group, x100 .
TEM- thin elongated muscles

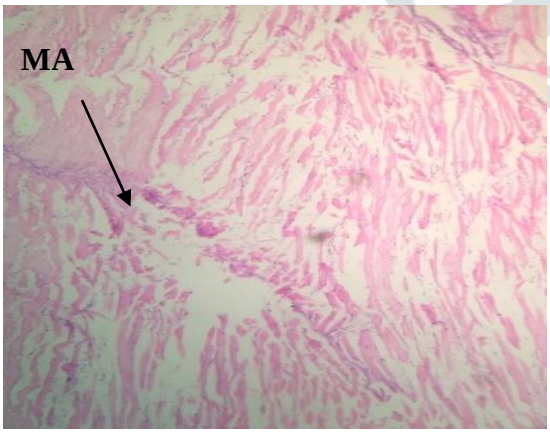

fig.9. sagittal section of muscles from 21 days of exposure group, x100.
MA- muscular atrophy

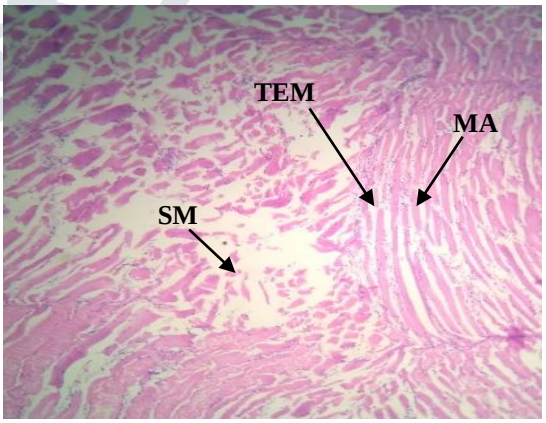

fig.10. sagittal section of muscles from 45 days of exposure group, x100.
TEM- thin elongated muscles, MA- muscular atrophy, SM- sparse muscles

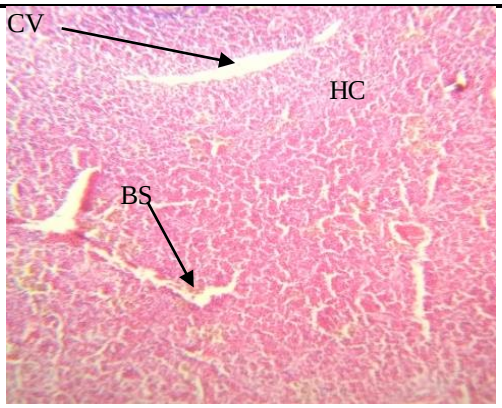

fig.11. sagittal section of liver from control group, x100 .
CV- central vein, H- hepatocytes, BS- blood sinusoids

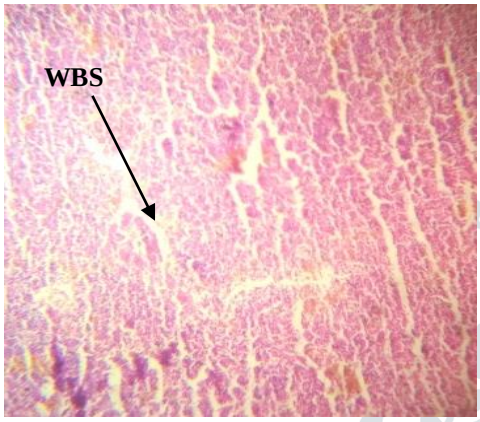

fig.12. sagittal section of liver from 7 days of exposure group, x100 .
WBS- wider blood sinusoids

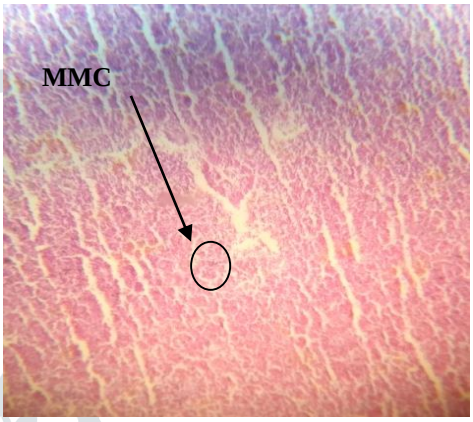

fig.13. sagittal section of liver from 14 days of exposure group, x100.
MMC- melanomacrophage centres

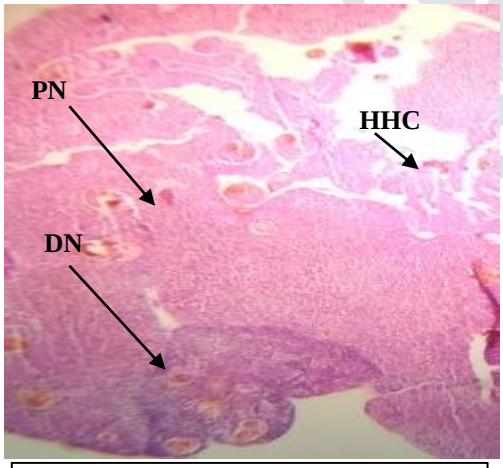

fig.14. sagittal section of liver from 21 days of exposure group, x100.
PN- pycnotic nuclei, HHC- hepatocyte hypertrophy, DN- degenerative nucleus

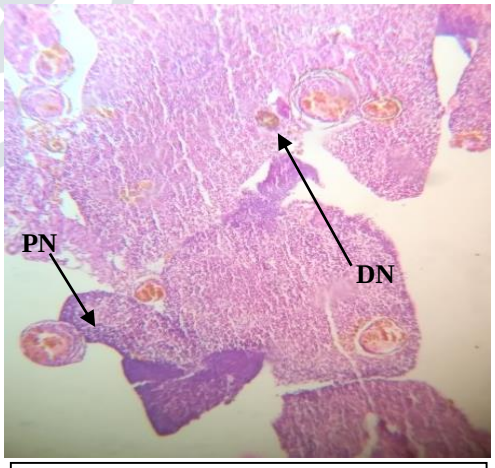

fig.15. sagittal section of liver from 45days of exposure group, x100.
PN- pycnotic nuclei, DN- degenerative nucleus

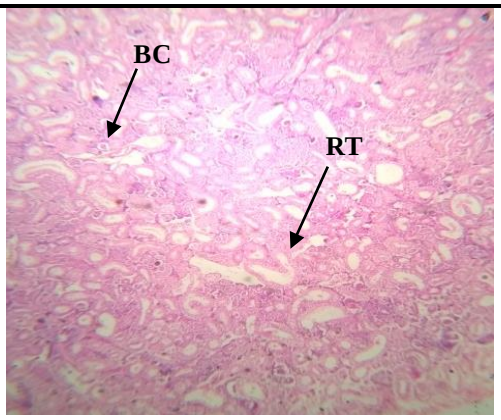

fig.16. sagittal section of kidney control group, x100.
BC- Bowman's capsule, RT- renal tubule

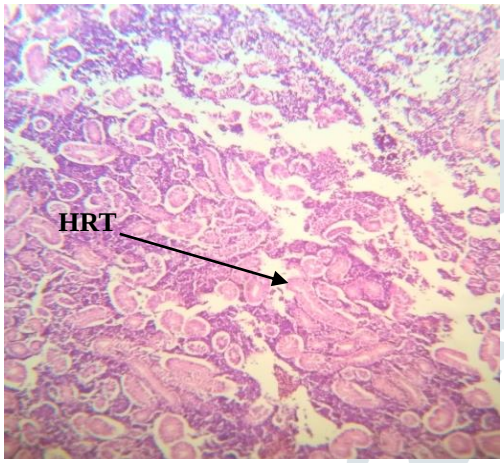

fig.17. sagittal section of kidney from 7 days of exposure group, x100.
HRT- hypertrophy of renal tubule

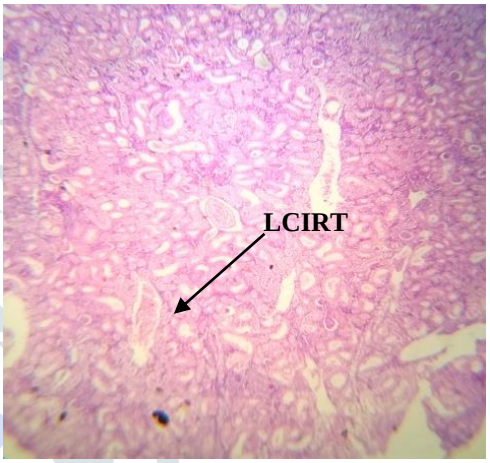

fig.18. sagittal section of kidney from 14days of exposure group, x100.
LCIRT- loss of cellular integrity of renal tubule

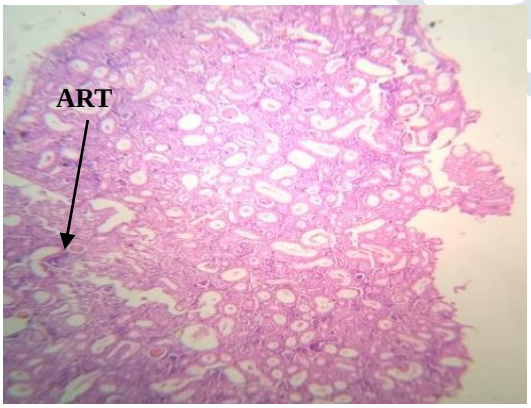

fig.19. sagittal section of kidney from 21days of exposure group, x100.
ART- atrophy of renal tubule

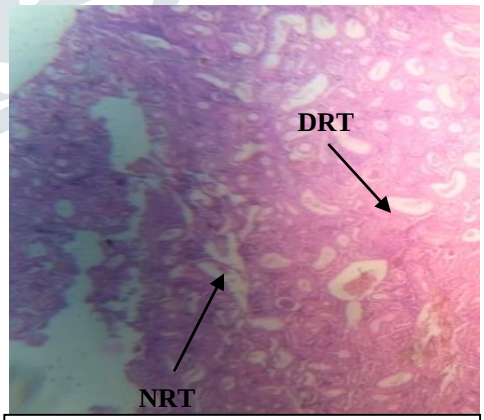

fig.20. sagittal section of kidney from 45days of exposure group, x100.
DRT- degeneration of renal tubule
NRT- necrosis of renal tubule

On previous occasions people have studied the histopathology of gills, liver, kidney, gonads etc. after inducing heavy metal toxicity in fishes. Lesions such as hyperplasia of epithelial cells covering the secondary lamellae, dilation of respiratory blood vessels have been recorded in the past after cadmium and lead toxicity induced in fishes [12, 13, 14].

Studies on Labeo rohita afetr lead nitrate toxicity revealed that thehistopathological effects goes on increasing in nearly all the tissues with increased doses and duration [15]. In the present investigations too similar changes were seen nearly in all the tissues. Dilated efferent branchiol artery, hyperplasia of gill lamellae and degenerations in the secondary lamellae were the major changes encountered histopathologically. Mucsles were affected due to the lead accettae treatment in the present investigations where muscle myotome appeared atrophid and elongated resulting into the loss of their integrity, tone and elasticity. These are

the common morphological changes found in fisnh muscle in response to the heavy metal toxicity. Chromium and manganese were reported to have similar effects on the muscle tissues [4,16]. Muscles seems to have maxium ability to store and magnify the heavy metals. Thus it is possible that such mrophological changes in fish muscles may cause impaired swimming movements. A report on the effect of lead nitrate on the liver of Labeo Rohita revealed degenerative changes which included necrosis, degerative nuclei and enfeltration of leucocytes in the hepatic synusoids etc [17,18]. Liver is the major organ of detoxification, it is a soft tissue which clears the toxicants and their metabolic products hence it is also a organ which undergoes maximum drug abuses. In the present investigation too we encountered similar histopathological alterations in the liver tissue.

Kidney is another organ invoved in the removal of toxicants, kidney in perticu;lar is the fore most organ to show histological damges, glomerular and tubular degeration enfiltration of edematous fluid [18]. Such changes were obsered even in the present investigation in all the treatment groups.

Heavy metals are known to accumulate in all types of tissues in nearly all the organisms and under go biomagnification with increasing trophic levels. Histopathological alterations studied under the current investigation also indicate at the damage caused by the lead to nearly all the vital organs. Our results are in concurrence with the results obtained by the previous studies.

IV. CONCLUSION

Heavy metal toxicity is known to affect all the organs adversely in a time dependent manner.A wide range of toxic effects of Lead (Pb) have been illustriously manifested in *Carassius auratus* in the present investigation. Histopathology though old, it is a very good indicator of toxic effects of any drug. The results obtained from histopathological studies can be correlated with biochemistry and behaviour of the experimental animals. From our observations it became clear that the gills and muscles get affected by the lead toxicity which may have caused respiratory stress leading to anoxic condition which further added stress on the skeletal muscles. Hyperplasia in skeletal muscles and those supporting the gills suggested loss of their functional integrity. This would affect the swimming and operculum movements. Abnormal rates of breathing can make the fish anoxic and set in the necrosis at the later stage. The results showed that the level of malformations and deformations of the tissues was dependent on the duration of the treatment. The clearance depot of the drugs which are kidney and liver were found to be degenerated in the present investigation, which is of a common occurrence under toxic effects caused by the heavy metals including lead.

References

[1] .V.R.Chavan and D.V.Muley (2014) : Effect of heavy metals on liver and gill of fish *Cirrhinusmrigala*, *Int.J.Curr.Microbiol.App.Sci, ISSN: 2319-7706* Volume 3 Number 5 (2014) pp. 277-288.

[2] Joshi, P. (2011): Impact of zinc sulphate on behavioural responses in the freshwater fish *Clariasbatrachus*(Linn.). *Online International Interdisciplinary Research Journal*, *1*(2), 76–82.

[3] Ashraf, M. A., Maah, M. J., &Yusoff, I. (2012). Bioaccumulation of heavy metals in fish species collected from former tin mining catchment. *International Journal of Environmental Research*, *6*(1), 209–218.

[4] SaravpreetKaur, Kuldeep Singh Khera and JasjitKaurKondal (2018): Heavy metal induced histopathological alterations in liver, muscle and kidney of freshwater cyprinid, *Labeorohita*(Hamilton), Journal of Entomology and Zoology Studies 2018; 6(2), E-ISSN: 2320-7078 P-ISSN: 2349-6800 : pp2137-2144.

[5] Perera, P. A. C. T., Kodithuwakku, S. P., Sundarabarathy, T. V., &Editrisinghe, U. (2015) : Bioaccumulation of cadmium on freshwater fish: An environmental perspective. *Insight Ecology*, *4*(1), 1–12.

[6] Saravpreet Kaur, Kuldeep Singh Khera and Jasjit Kaur Kondal (2018): Effect of water contaminated with heavy metals on histopathology of freshwater catfish, *Clarias batrachus*, International Journal of Chemical Studies ; 6(4): 3103-3108.

[7] Järup L. (2003): Hazards of heavy metal contamination. British Medical Bulletin. 68(1):167-182.

[8] Szentmihalyi K, Then M. (2007): Examination of microelements in medicinal plants of carpathion basin. Acta Aliment.; 36:231-236.

[9] SeharAfshan, Shafaqat Ali*, UzmaShaistaAmeen, MujahidFarid, SaimaAslamBharwana, FakhirHannan, Rehan Ahmad (2014): Effect of Different Heavy Metal Pollution on Fish Res. J. Chem. Env. Sci., Volume 2 Issue 1 February 2014, Online ISSN 2321-1040: pp 74-79.

[10] APHA (2005).Standard Methods for the Examination of Water and Wastewater. 21st Centennial edi-tion ,American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF), Washington DC, USA.

[11] Finney, D.J. (1971). Probit Analysis. 3rd Edition, Cambridge University Press, London, 330 pp.

[12] Banerjee TK & Chandra S (2005): Estimation of zinc chloride contamination by histopathological analysis of therespiratory organs of the air breathing "murrel" *Channa striatus* (Bloch, 1797) (Channiformes, pisces). *Vet Arhiv*, 75 (2005) 253.

[13] .Olojo EAA, Olurin KB, Mbaka G & Oluwemimo AD (2005): Histopathology of the gill and liver tissues of the African catfish *Clarias gariepinus* exposed to lead *Biotechnol*, 4 (2005) 117.

[14] Brraich OS & Kaur M (2017): Histopathological alterations in the kidneys of *Labeo rohita* due to lead toxicity. *J Environ Biol*, 38 (2017) 257.

[15] Onkar Singh Brraich & Manjeet Kaur (2017) : Histopathological alterations in the gills of *Labeo rohita* (Hamilton-Buchanan) due to Lead toxicity, Indian Journal of Experimental Biology Vol. 55, August 2017, pp. 576-583.

[16] SitiNadzirah Padrilah1, MohdKhalizan Sabullah2, MohdYunusAbd Shukor1, NurAdeela Yasid1, NorAripin Shamaan3 and SitiAqlima Ahmad (2018): Toxicity Effects of Fish Histopathology on Copper Accumulation, Pertanika J. Trop. Agric. Sci. 41 (2): 519 – 540.

[17] Justina I. R. Udotong (2015): Histopathological Changes in Liver and Muscle of Tilapia Fish from QIRE Exposed to Concentrations of Heavy Metals, World Academy of Science, Engineering and Technology, International Journal of Biotechnology and Bioengineering,Vol:9, No:6

[18] A. Hadi and S. F. Alwan (2012): Histopathological changes in gills, liver and kidney of fresh water fish, Tilapia zillii, exposed to aluminium, INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES, ISSN: 0976-7126, 3(11).