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Monitoring the Effect of Lead Acetate on Histopathological Changes in *Barbus Sharpeyi*

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Abstract: The present study was carried out to determine the effect of lead acetate on histopathological changes in Bunni (Barbus sharpeyi), as well as the description of fish behavior. A total of 300 fingerlings were used in the laboratory of fish diseases in the College of Veterinary Medicine - University of Baghdad for the period between 1/3 to 1/6/2013. Fish were distributed randomly into four treatments in addition to control group. First treatment (T1) contained lead acetate 0.42 mg/l with replacement water aquarium entirely per two days and added lead acetate continuously, the second treatment (T2) contained lead acetate 0.42mg/l with replacement water aquarium entirely per two days without adding lead acetate, third treatment (T3) contained lead acetate 0.21mg/l with replacement water aquarium entirely per two days and adding lead acetate continuously, fourth treatment (T4) contained lead acetate 0.21mg/l with replacement water aquarium entirely per two days without adding lead acetate. In order to estimate LC50 used 120 fingerlings of B. sharpeyi, were exposed to 1, 2, 3, 4, 5 and 6 mg/l. The LC50 of lead acetate was 4.24 mg/l for 72h. of exposure. Fish behavior showed abnormalities after exposure to the various lead acetate concentrations such as swimming disorders, the fish tended together at the surface, fast movement, aggregate in aquarium border, weakness, with increasing in the speed of movement of the operculum, of T1,T2 and T3 and decreasing in feeding process in T1 and T3. Histopathological changes were detected in gills characterized by lamellar fusion, lifting of secondary lamella. The main findings in liver tissue are hydropic swelling and fatty degeneration of some hepatocytes, focal or diffuse necrosis. Kidney showed varying degrees of tubular necrosis with severe congestion together with melanomacrophage infiltration. Spleen observed severe destruction in spleen, parenchyma, and severe reduction in hemopoitic tissue.

Keywords: *Barbus sharpeyi,* Lead Acetate, Histopathology

1. Introduction

The element lead (Pb) is probably the most extensively studied of ″heavy metals″ contaminant, due to its ubiquitous occurrence in the environment and it's probably adverse neurological and health effects (Melgar et al., 1997). The major sources of lead resulting from human activities, such as sewage sludge, painting, paint industries, fuel incinerator, plumbing supplies, potential anthropogenic sources of air borne lead, source as mining, smelting, coal combustion, waste incineration and the use of Pb additives in fuel, exceed the contribution of natural sources on the global scale (Bellis et al., 2001). When the concentrations of lead metal exceed the normal limit, it become poisonous and may be fatal to humans and other organisms (Niebaer and Richardson, 1980). The organ lead compounds tetraethyl and tetramathyl. Lead can accumulate in some organs of fish such as liver and kidneys and cause lethal and a variety of sub lethal effects (Ozmen et al., 2006). Kock *et al.* (1996) showed that lead levels in *Salvelinus alpinus* liver and kidneys indicate higher uptake rates of both in summer when water temperature was higher. Bioaccumulation and magnification is capable of leading to toxic level of these metals in fish even when the exposure is low. The aim of the present study is to estimate the median lethal concentration of the lead metal in commercial fish *Barbus sharpeyi* and to detect clinical signs in such fish after exposure to lead metal such as movement, feeding, growth and mortality, as well as studying the histopathological changes in fish body organs (gill, kidney, liver, and spleen).

2. Materials and Methods

This study was conducted at the laboratory of fish diseases in the College of Veterinary Medicine, University of Baghdad for the period between 1/3 to 1/6/2013. A total of 300 fingerlings of Bunni fish (*B. sharpeyi*) ranging between 10-15g in body weight, with no visible signs of disease were used. Fish were brought from Al-Swerra hatchery and acclimated to laboratory condition for 15 days before beginning of the experiment. Fish were briefly bathed in NaCl for 5min to remove all external parasites if present.

To determine the median lethal concentration (LC_{50}) of lead acetate, 6 treatments were used each treatment contained 10 fishes and each was transferred to 70 L of water, as well as control group without adding lead , with two replicate for each treatment. Six different concentrations of lead acetate were used; 1mg/l, 2 mg/l, 3 mg/l, 4mg/l, 5 mg/l and 6 mg/l, respectively

The concentration at which 50% mortality of fishes occurred after 72hrs was selected as the median lethal concentration (LC_{50}). The LC_{50} concentration for 72hrs was calculated by the probit analysis method according to Goldstein *et al.*(1974).

During the experiment period the observations of toxic symptoms such as stress, movement, food intake, respiration, swimming, responses to the outer effects were recorded. Body weight was measured before and after the experiment for any alteration during the experiment period.

Histopathological changes were studied in fish that exposed to lead acetate. After dissection, samples from gills, liver, kidney and spleen were collected per fish and fixed in 10% formalin for 24h, dehydrated in graded ethanol concentrations and embedded in paraffin wax. Sagittal sections (5µm of thickness) were cut and mounted on glass slides. Sections were deparaffinized in xylene, hydrated in ethanol, stained with hematoxylin – eosin (HE) and examined by light microscope (Luna, 1968).

3. Results and Discussion

Probit method was applied to estimate of LC_{50} for lead acetate of *Barbus sharpeyi* as shown in table (1). The present study determined the LC_{50} of lead acetate as 4.24mg/l, LC_0 (1mg/l) and LC_{100} (7mg/l).In the acute toxicity test, approximately 1 h after exposure for the various lethal lead acetate concentrations, the fishes showed abnormal behaviors included increase movement, frequent jumping, erratic swimming, convulsion, and escape attempts from the aquarium, loss of equilibrium. Results showed no mortality of fishes in the control groups. The effect of acute toxicity of lead acetate concentrations on *Barbus sharpeyi* at different exposure period and the mortality percentages are shown in Fig. (1).

Table 1. *Lead acetate concentrations and mortality of Barbus sharpeyi.*

Conc. mg/ L	Log Conc.	Fish No.	Survival Fish	Mortality	Mortality %	Corrected Mortality%	Probit No.
Control	…….	10	10	0	0	……..	……..
1	0	10	10	0	0	2.5	2.95
2	0.30	10	9	1	10	10	3.72
3	0.47	10	8	2	20	20	4.16
4	0.60	10	6	4	40	40	4.75
5	0.70	10	4	6	60	60	5.25
6	0.77	10	2	8	80	80	5.84

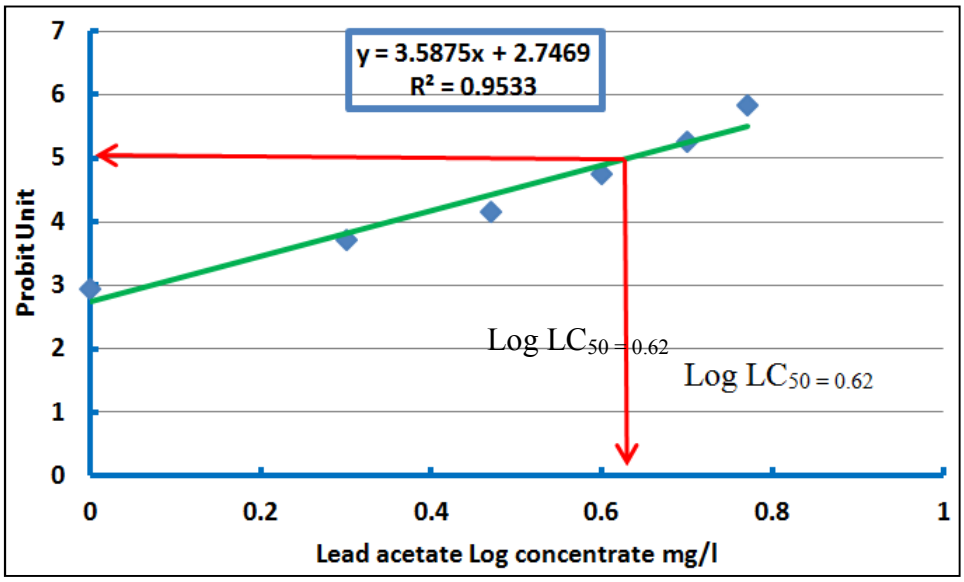

Fig. 1. *Linear relationship between response (probit units) and log concentration of Lead acetate at 48h.*

The mortality of the fish increased along with increasing the lead acetate concentrations and length of exposure periods. At the low $PbHCO_3$ concentration (2 and 3 mg/l), mortality detected after 72 h of exposure, while at higher concentrations (5 and 6mg/l), the mortality observed after 24 h of exposure.

The present study determined the acute toxicity of lead acetate on *B. sharpeyi* during 48 h. The LC_{50} of lead acetate was 4.24mg/l. The concentrations of lead in the marine and freshwater environment have been considerably increased by human activities. The rate of introduction of lead into the water increased 27 fold since the pleistocene period (Tatsumoto and Patterson, 1963).

The exposure to heavy metals can affect reproduction efficiency of aquatic biota and can lead to a gradual distinction of their generations in polluted waters (Ebrahimi, 2004). Heavy metal contamination not only directly affects fish health, but it can also disrupt the normal steroidogenesis pattern in fish, lead to impaired hormone production in both male and female fish, and decrease the quality and quantity of sperm and ova production. (Enrique et al., (2002); Munoz et al., 2005).

The effect of the metal also depends on the species and size of the fish, salinity of water, water temperature, pH and exposure time. The better water temperature in the rearing aquarium ranged between 24-26°C. Temperature is an important factor, which regulates the biogeochemical activities in the aquatic environment such as fish (Boyd, 1990).

Generally fish behavior showed abnormalities approximately 1h. after exposure to the various lead acetate concentrations such as, increase swimming activity, hypersensitivity, increase operculum movement. During the experiment a few fishes start drowning by sudden somersaulting, regain normal posture and balance temporarily. Finally, however, they succumb to poison with mouth and operculum wide open, the changes in behavior are conspicuous, and paralysis. Results showed fish loss appetite of food intake.

Loss of equilibrium follows erratic and darting swimming movements, which might be due to the inhibition of brain cytochrome C oxidase activity, causing cytotoxic hypoxia, thus causing brain damage to the region of the brain associated with the maintenance of equilibrium (Nsikak et al., 2007).

The oxygen consumption endpoint also provides an index for sub-lethal stress and for bio-monitoring the potentially toxic effects of chemicals

The rates at which the swimming behavior appeared, depends on the species of fish and sensitive to low Dissolved Oxygen levels. DO also regulate the availability of certain nutrients in water. Many physical and biological factors affect the amount of dissolved oxygen in river (El-Nemaki et al., 2008).

Salinity also reduces the solubility of oxygen (Karlesen et al., 2000; Al-Saffar, 2006), in addition to that biological process of photosynthesis and respiration also affect DO concentration in river. As aquatic plant photosynthesis, they give off large amount of DO during day light hours. However, respiration from aquatic vegetation, microorganisms, and algae consume oxygen at all hours of the day and night, the fluctuation occurs in the dissolved oxygen levels (Sivakumar and Karuppasam, 2008).

Fish exposed to T1, (0.42mg/l of lead acetate), the gills showed severe congestion in lamellar capillaries and Venus nervous sinus accompanied with sever hyperplasia of mucous cells in the secondary lamellar epithelia that filled with mucous substances together with scatter proliferation of chloride cells mainly at the base lamella (Fig.2) accompanied with severe vacillation with cystic space formation in the epithelial of secondary lamella associated with mononuclear cells infiltration (Fig.3). In T2 (0.42 mg/l) the histological appearance of gill lesion characterized by mononuclear cells infiltration in the secondary lamella with slight epithelial hyperplasia of secondary lamella epithelium (Fig.4). Fish exposed to T3 (0.21mg/l lead acetate) the predominant lesion in gill tissue at this treatment showed slide circulation of secondary lamella with slight epithelial hyperplasia (Fig.5) other section showed focal secondary lamellaer fusion accompanied with slight lamellar epithelial lifting (Fig.6) Fish exposed to T4 (0.21 mg/l lead acetate) showed no clear pathological changes except MNCs aggregation in central venus sinus.

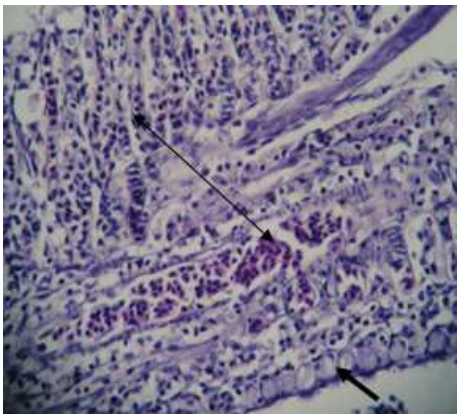

Fig. 2. *Gills section of T1 shows severe congestion in lamellar capillaries and central venus sinus (↔) with sever hyperplasia of mucous cells in the secondary lamellar epithelia (→) (H&Ex40).*

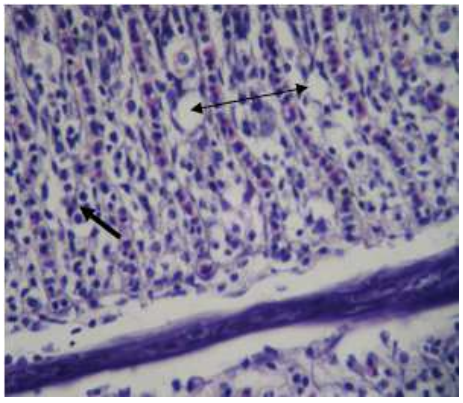

Fig. 3. *Gills section of T1 group shows moderate vaculation in the epithelial of secondary lamella (↔) with MNCs infiltration (→) (H&Ex40).*

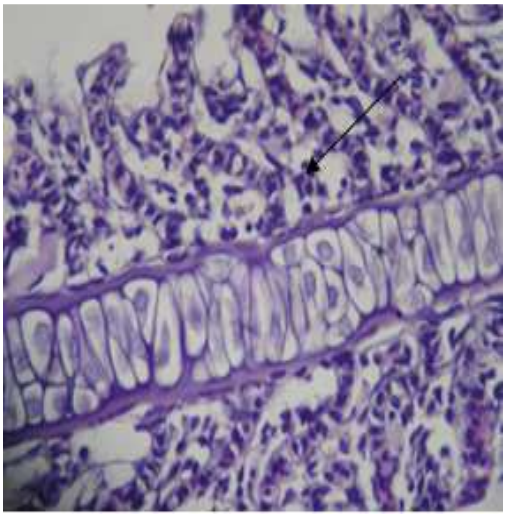

Fig. 4. *Gills section of T2 shows diffuse MNCs infiltration in the secondary lamella (→) (H&Ex40).*

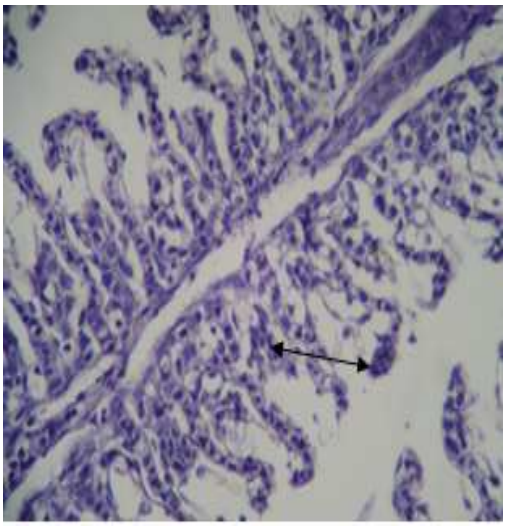

Fig. 5. *Gills section of T3 shows slight circulation of secondary lamellae with slight epithelial hyperplasia (↔) (H&Ex40).*

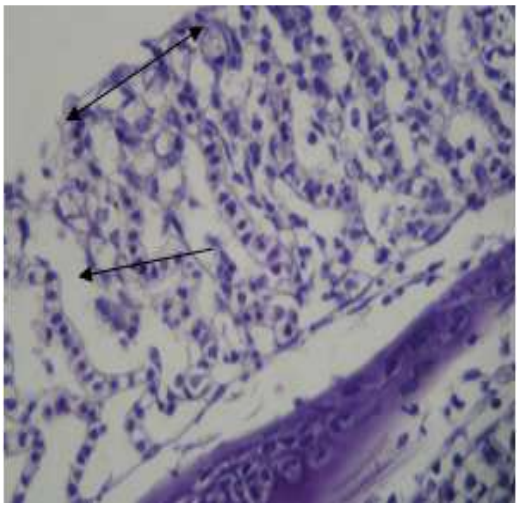

Fig. 6. *Gills section of T3 shows focal secondary lamellar fusion (↔) with slight epithelial lamellar lifting (→) (H&Ex40).*

The kidney sections at T1(0.42 mg/l) showed tubular changes varied between vacuolar changes of tubular epithelial lining to sever tubular necrosis accompanied with present basophilic bacterial colonies (Fig.7). The kidney sections exposed to T2(0.42 mg/l) concentration of lead acetate showed interstitial mononuclear cells infiltration consist mainly macrophage and lymphocyte accompanied with sever hydropic swelling of tubular epithelial lining(Fig.8) , other section showed sever hemorrhage and congestion in kidney parenchyma of this treatment with tubular degeneration varied between cellular swelling and hydropic changes in other tubules (Fig.9). At T3 (0.21mg/l) sever depletion of hemopoitic tissues with glomerular tuft vacular (Fig.10).

The kidney sections At T4 (0.21 mg/l) showed interstisial MNCs infiltration consist mainly of macrophage and plasma cells associated with slight cellular swelling (Fig.11).

The specific structural lesions observed in the liver parenchyma exposed to T1 (0.42 mg/l) were massive destruction in liver parenchyma resulting in sever vacillation with necrosis of hepatocyte associated with nuclear pyknosis (Fig.12) also the results show sinusoidal dilation and congestion in other section with periductal MNCs infiltration (Fig. 13).

At T2, (0.42 mg/l) the fish liver showed moderate cellular swelling of hepatocytes of this treatment with slight sinusoidal congestion associated with slight proliferation of tissue macrophage (Fig.14) At T3 (0.21 mg/l), the fish liver showed sever liver necrosis with sinusoidal congestion together with slight hyperplasia of bile duct (Fig.15) associated with degenerative and necrotic changes were recorded in liver parenchyma, characterized by distribution in hepatic cord with individual hepatic cell apoptosis (Fig.16).

At T4 (0.21 mg/l), no clear pathological changes observed except bile duct dilation with MNCs infiltration (Fig.17). The hall mark of spleen tissue exposed to T1 (0.42 mg/l) characterized by severe destruction in both spleenic tissue with spleenic sinus congestion together with variable number of melanomacrophage in addition to lymphoid depletion in white pulp (Fig.18) at (T2) (0.42 mg/l), the main lesion characterized by melanomacrophage hyperplasia that appear as brown golding cluster (Fig.19) .

At T3, (0.21 mg/l), the fish spleen appeared spares distribution of melanomacrophage cells mainly around sinuses (Fig.20) associated with moderate to severe lymphoid depletion as well as slight vacillation mainly near the sub capsular lesion (Fig.21).

At T4 (0.21mg/l), the fish spleen no pathological lesion was observed except slight congestion of spleenic sinuses (Fig.22).

The gill histopathological finding in the present study is similar to the observation obtained by Triebskorn et al., (2008) that the basic gill pathology, hyperplasia, detected in the majority of studied fish, and mild to severe hyperplasia detected in almost every fish from the basin of the lower Amur River. Hyperplasia is regarded as a nonspecific defense reaction to heavy metals and mixed pollution and has been described in many reports.

Appearance of inflammatory cells in the gill tissue (moderate to marked) and all these alteration could represent a defense mechanism of the body to increase the distance across which waterborne pollutants must diffuse to reach the blood stream (Arellano et al., 1999). The liver histopathological finding in the present study similar finding were revealed in sleek Unicorn fish *Naso hexcanthus* exposed to heavy metal at contaminated site in the red sea areas (Montaser et al., 2010). The histological alterations of hepatocytes identified in this study may be the result of various biochemical lesions and act as a signal of degenerative processes that suggests metabolic damage (Pacheco and Santos, 2002). Alterations of kidney tissue during the acute exposure were severe. They were composed principally of tubule necrosis, glomerular alteration and lipid inclusion accumulation in epithelial cells. Following the chronic contamination, severe glomerular alteration was noted in this tissue.

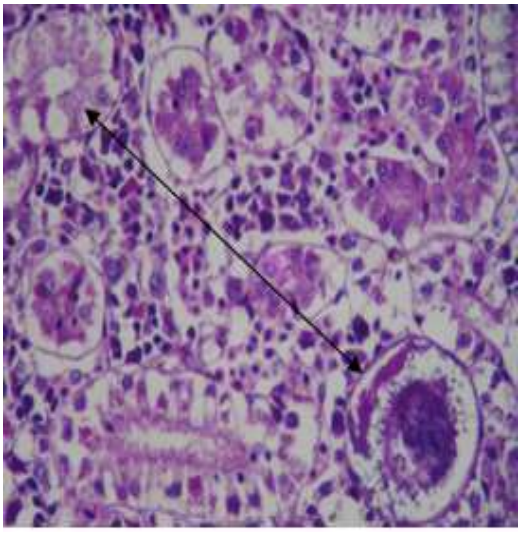

Fig. 7. *Kidney section of T1 shows severe hydropic swelling of tubular epithelial lining with necrosis in other tubules (↔) (H&Ex40).*

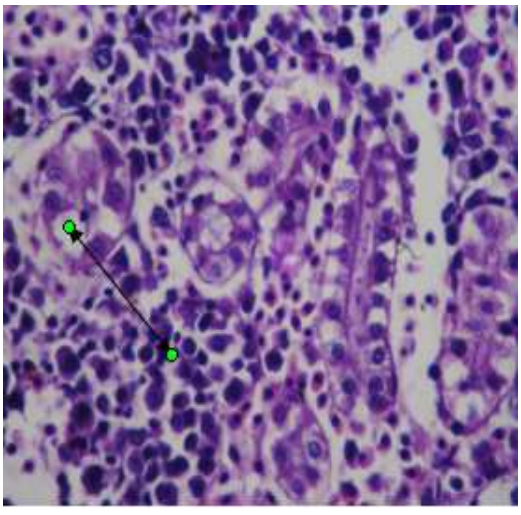

Fig. 8. *Kidney section of T2 shows severe hydropic swelling with MNCs infiltration (↔) (H&Ex40).*

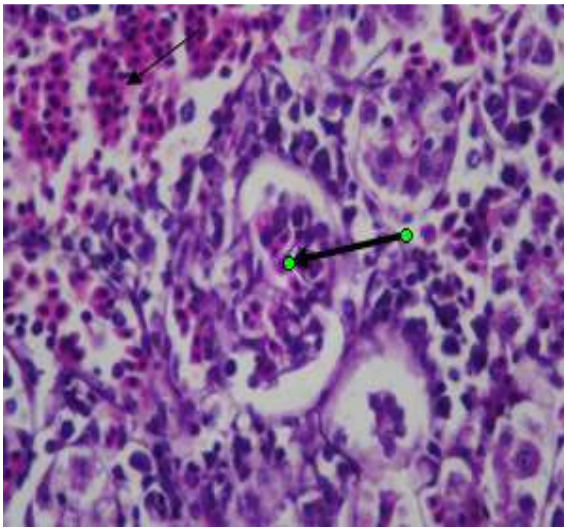

Fig. 9. *Kidney section of T2 shows congestion in kidney parenchyma (→) with cong. of glomerular tuft (↔) (H&Ex40).*

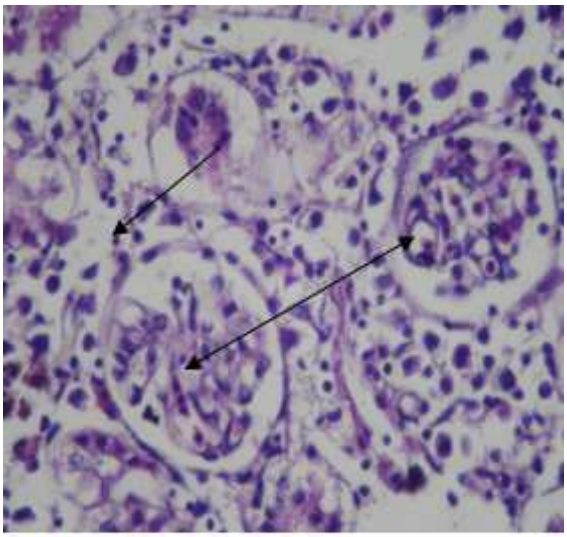

Fig. 10. *Kidney section of T3 shows severe depletion of hemopiotic tissues (→) glomerular tuft vacullation (↔) (H&Ex40).*

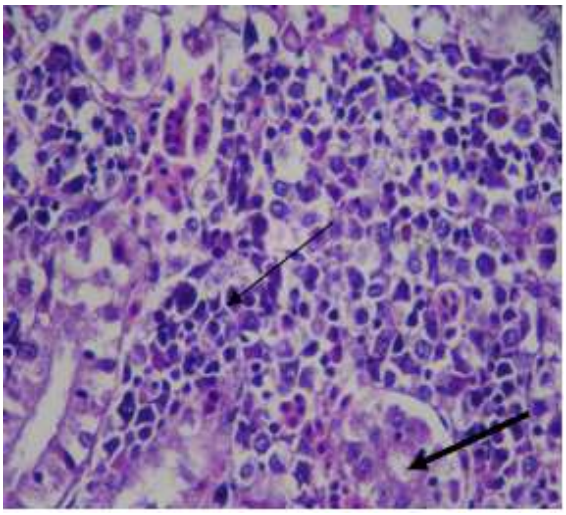

Fig. 11. *Kidney section of T4 shows MNCs infiltration (→) with slight cellular swelling (→) (H&Ex40).*

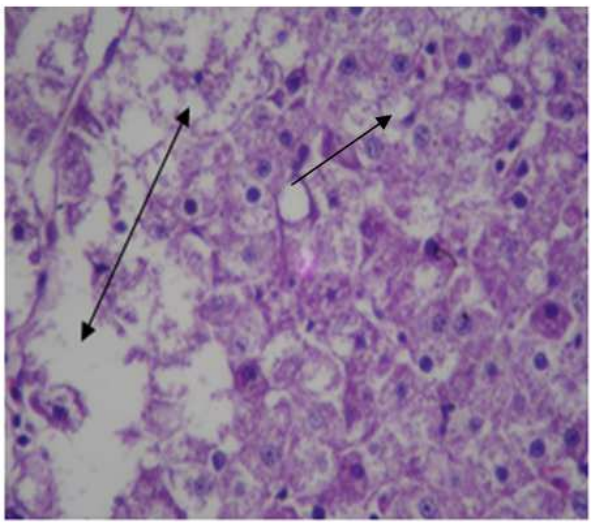

Fig. 12. *Liver section of T1 shows severe vacillation (↔) with necrosis of hepatocyte (→) (H&Ex40).*

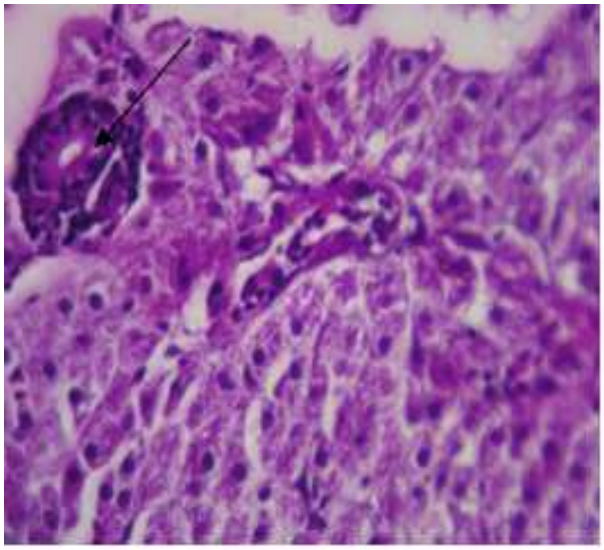

Fig. 13. *Liver section of T1 shows periductal MNCs infiltration (→) (H&Ex40).*

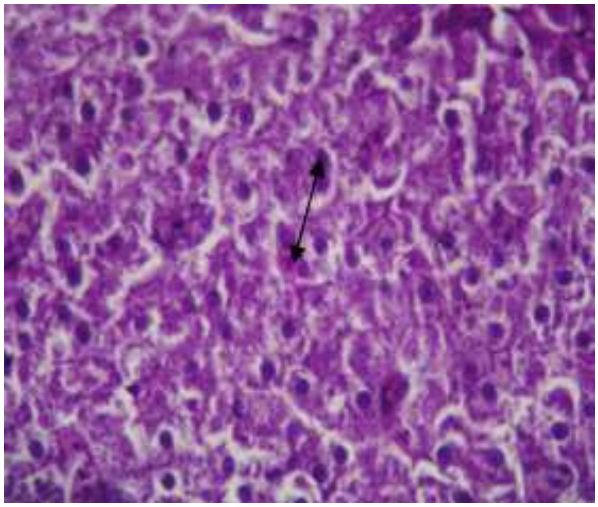

Fig. 14. *Liver section of T2 shows moderate cellular swelling of hepatocytes with slight sinusoidal congestion (↔) (H&Ex40).*

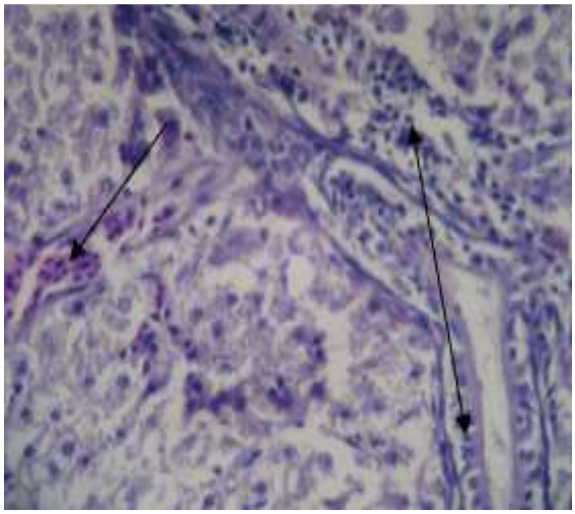

Fig. 15. *Lliver section of T3 shows sinusoidal congestion & dilation (→) with slight periductal MNCs aggregation and ductal dilation (↔) (H&Ex40).*

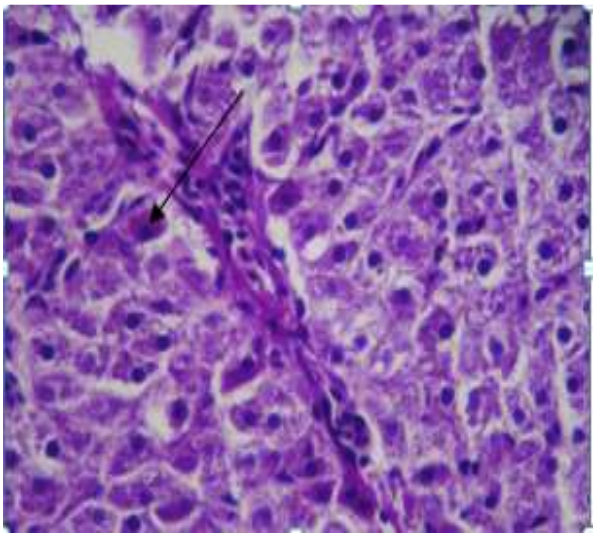

Fig. 16. *Liver section of T3 shows hepatic cell apaptosis (→) (H&Ex40).*

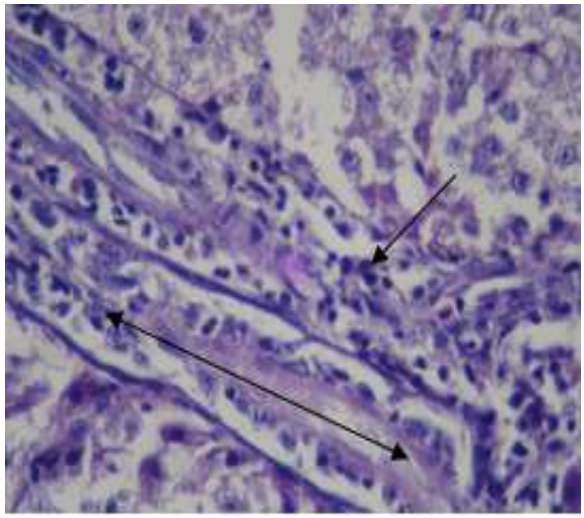

Fig. 17. *Liver section of T4 shows MNCs infiltration (→) bile duct dilation (↔) (H&Ex40).*

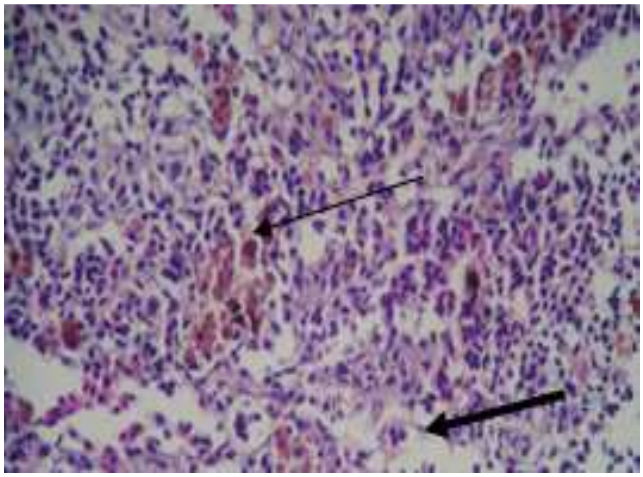

Fig. 18. *Spleen section of T1 shows discrete melanomacrophage (⟶) lymphoid depletion in white pulp (⟶) (H&Ex20).*

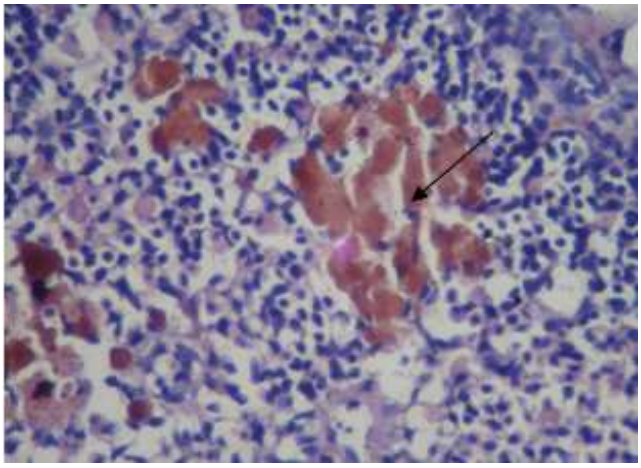

Fig. 19. *Spleen section of T2 shows melanomacrophage hyperplasia (brown golding cluster) (⟶) (H&Ex40).*

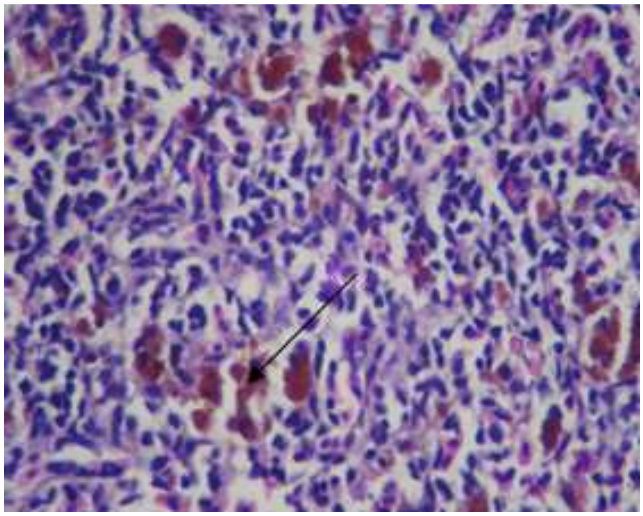

Fig. 20. *Spleen section of T3 shows melanomacrophage mainly around splenic sinuses (⟶) (H&Ex40).*

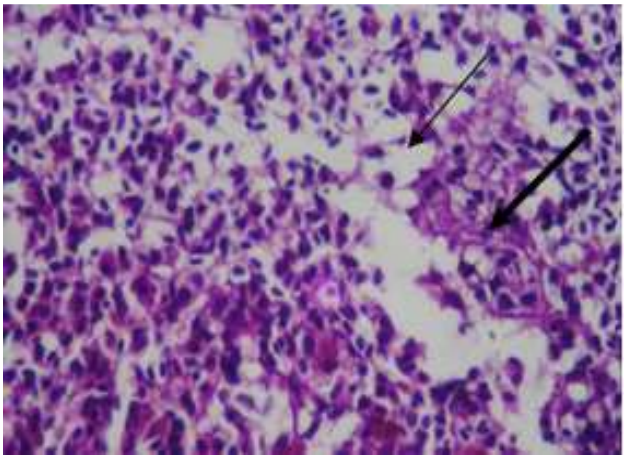

Fig. 21. *Spleen section of T3 shows vaculation (⟶) with lymphoid depletion (⟶) (H&Ex40).*

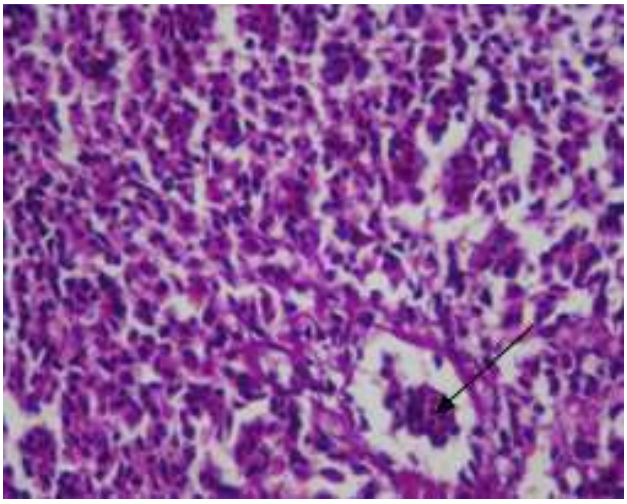

Fig. 22. *Spleen section of T4 shows slight congestion of splenic sinuses (⟶) (H&Ex20).*

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