Histopathological Effects of Cadmium Chloride on *Barbus sharpeyi*

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Abstract

The present study including determined pathological changes in *Barbus sharpeyi*, as well as description behavior and growth of bunni fish by used 180 fingerlings, Fish were distributed randomly upon four treatments in addition to control group. First treatment (T1) contained cadmium 0.093mg/L with changing water and added cadmium continuously, the second treatment (T2) contained cadmium 0.093mg/L with changing water without adding cadmium, third treatment (T3) contained cadmium 0.046mg/L with changing water and adding cadmium continuously, fourth treatment (T4) contained cadmium 0.046mg/L with changing water without adding cadmium. In order to estimate LC50 used 120 fingerlings of fish *B. sharpeyi*, were exposed to 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0mg/L. The LC_{50} of cadmium was 0.932mg/L for 48h of exposure. Fish behavior was recorded showed abnormalities after exposure to the various cadmium concentrations such as swimming disorders, the fish tended together at the surface, fast movement, fish aggregate in aquarium border, fish weakness, opened and closed in operculum festally, as well as a significant decrease at ($P \leq 0.05$) in body weight of T1,T2 and T3. Histopathological changes in gills characterized by lamellar fusion, lifting of secondary lamella, The main findings in liver tissue were hydropic swelling and fatty degeneration of some hepatocytes, focal or diffuse necrosis, Kidney result varying degrees of tubular necrosis with severe congestion together with melanomacrophage infiltration. Intestine results showed slauphing and necrosis of mucosal epithelial of intestinal villi and diffuse MNCs infiltration, hyperplasia and hypertrophy of goblet cell. Spleen observed severe destruction in spleenic parenchyma, severe reduction in hemopoitic tissue.

Keywords: Cadmium Chloride, Histopathology , *Barbus sharpeyi.*

التأثيرات النسجية المرضية لكلوريد الكادميوم في اسماك البني *Barbus sharpeyi*

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الخلاصة

هدفت الدراسة الحالية معرفة التأثيرات المرضية في اسماك البني ووصف التغيرات السلوكية ونمو الاسماك باستعمال 180اصبعية من اسماك البني، قسمت الى اربع معاملات فضلا عن معاملة السيطرة ، المعاملة الاولى احتوت على الكادميوم بتركيز 0.093 ملغم/لتر مع تبديل ماء الحوض كلياً واضافة الكادميوم ، اما المعاملة الثانية احتوت على الكادميوم بتركيز 0.093 ملغم/لتر مع تبديل ماء الحوض كلياً باستمرار دون اضافة الكادميوم،

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والثالثة بتركيز 0.046 ملغم/لتر مع تبديل ماء الحوض كلياً واضافة الكادميوم، والرابعة بتركيز 0.046 ملغم/لتر مع تبديل ماء الحوض كلياً باستمرار دون اضافة الكادميوم. أستعمل 120 أصبعية من أسماك لغرض تحديد التركيز المميت الوسطي وذلك بتعريض الأصبعيات للتراكيز الأتية (0.5، 0.6، 0.7، 0.8، 0.9، 1.0 ملغم/ لتر)وكان التركيز المميت الوسطي 0.932 ملغم/لتر خلال 48 ساعة. شملت الدراسة وصف التغيرات في سلوك الأسماك المعاملة اذ أختلف سلوكها حسب تركيز الكادميوم في الحوض وتمثلت بالسباحة بصورة عمودية متجهة لاسفل الحوض والسباحة على سطح الماء والحركة السريعة للاسماك وتجمع الاسماك في احدى زوايا الحوض وزيادة حركة الغطاء الغلصمي.وأظهرت نتائج التحليل الأحصائي أنخفاصاً معنوياً بمستوى ($P\leq0.05$) في معدل أوزان الأسماك في المعاملات الاولى والثانية والثالثة. تميزت التغيرات النسجية في الغلاصم بأندماج وألتصاق الصفائح الغلصمية أضافة الى تلف الخلايا في ظهارة الصفائح الغلصمية الثانوية. أما نسيج الكبد فتميز بوجود التورم الأستسقائي مصحوباً بالتنكس الدهني و نخريؤري منتشر. أما الكلية فأظهرت درجات متفاوتة مابين التنكس النبيبي وحدوث النخر المصحوب بأحتقان الأوعية مع أرتشاح الخلايا البلعمية الميلانينية. أما الأمعاء فتميزت بانسلاخ وتنخرالمخاطية الظهارية من الزغابات المعوية وارتشاح الخلايا وحيدة النواة وتضخم وزيادة الخلايا الكأسية.أما نسيج الطحال فأظهرتحطم في خلايا الطحال ونفاذ اللمف في اللب الابيض واختزال في الانسجة الدموية.

Introduction:

Heavy metals are natural trace components of the aquatic environment, but background levels have increased due to industrial wastes, agricultural and mining activities [1].

In an aquatic environment, metal toxicity can be influenced by various abiotic environmental factors such as oxygen, hardness [2], pH, alkalinity and temperature [3]. In fishes, apart from the environmental factors, metal toxicity is also affected by the length and weight of fishes, season, feeding [4] and time of exposure to metals [5]. Cd can accumulate in some organs of fish causing lethal or sub lethal effects [6]. Among these toxic substances, heavy metals constitute one of the main dangerous groups, they are toxic, persistent and not easily biodegradable [7, 8]. Cadmium is heavy metal and poses high toxicity at very low level of exposure. Long exposure to cadmium produces a wide variety of acute and chronic effects in aquatic animals. Its prime site is kidney [9, 10]. The aims of the present study include: Measuring the Median Lethal Concentration of the Cadmium Chloride metal in *Barbus sharpeyi*. Assess clinical signs in *B. sharpeyi* after exposure to cadmium metal (movement, feeding, growth and mortality). And studying the histopathological changes in fish organs (gill, kidney, liver, intestine and spleen).

Materials and Methods:

This study was conducted at the College of Veterinary Medicine, University of Baghdad, Ichthyology Laboratory. A total of 300 fingerlings of Bunni fish *B. sharpeyi* ranging between 8-10 cm in total length and 10-15gm in body weight, with no visible signs of disease or morbidity, were obtained 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 for remove all external parasite if present.

To determine the median lethal concentration (LC_{50}) of cadmium chloride, 6 treatments were used each treatment contained 10 fishes and each was transferred to 70 L of water. Two control treatments of fish were also established, both with water only. Six different concentrations of cadmium chloride were used; 0.5mg/L, 0.6 mg/L, 0.7 mg/L, 0.8 mg/L, 0.9 mg/L and 1.0 mg/L, the concentration at which 50% mortality of fishes occurred after 48hrs was selected as the median lethal concentration (LC_{50}). The LC_{50} concentration for 72hrs was calculated by the probit analysis method.

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

Histopathological changes were studied in fish that exposed to cadmium chloride. After dissection, samples from intestine, 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.

Results and Discussion:

The estimated LC_{50} by (Probit method) for cadmium chloride of *Barbus sharpeyi* is shown in Table-1. In the acute toxicity test, approximately 1 h after exposure to the various lethal cadmium chloride concentrations, the fishes showed behavioral abnormalities such as: 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 acute toxicity of cadmium chloride concentrations on *Barbus sharpeyi* at different exposure period and the mortality percentages are shown in Figure-1.

The mortality of the fish increased with cadmium chloride concentrations and length of exposure times. At the low cadmium chloride concentration (0.6 and 0.7 mg/L), mortality appeared after 72 h of exposure, While at higher concentrations (0.9 and 1mg/L), mortality occurred after 24 h of exposure. The present study determined the LC_{50} of cadmium chloride (0.932mg/L), LC_0 (0.5mg/L) and LC_{100} (1.4mg/L).

Table 1- LC_{50} of Cadmium Chloride on *Barbus sharpeyi* by Probit method.

Conc. mg/L	Log Conc.	Fish No.	Fish survival	Mortality	Mortality %	Corrected mortality%	Probit No.
Control	…….	10	10	0	0	……..	……..
0.5	2.69	10	10	0	0	2.5	2.95
0.6	2.77	10	9	1	10	10	3.72
0.7	2.84	10	8	2	20	20	4.16
0.8	2.90	10	7	3	30	30	4.48
0.9	2.95	10	6	4	40	40	4.75
1.0	3.00	10	4	6	60	60	5.25

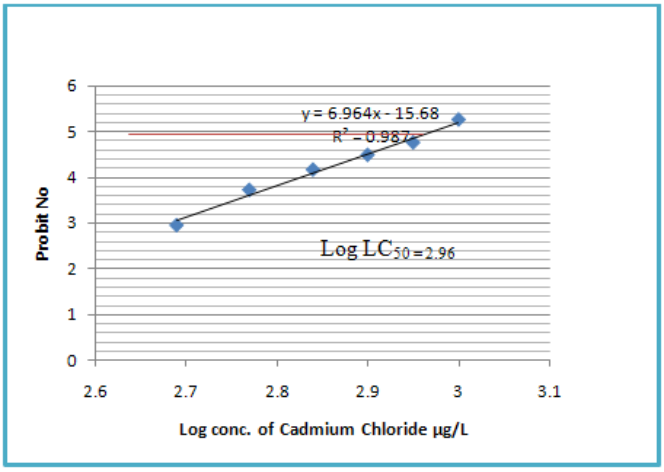


Figure 1-Linear relationship between probit response and log concentration of median lethal concentration for cadmium chloride in 48h.

The present study determined the acute toxicity of cadmium chloride on *B. sharpeyi* during 48 h. The LC_{50} of cadmium chloride was 0.932mg/L. In another study, [11] reported LC_{50} values of about 0.3 to 50 mg Cd /L for various marine fish. The calculated average 96-hr LC50 is 4.533 mg/L in freshwater fish, *Catla catla* [12] , LC_{50} has been established in 25 mg/L for scorpion fish, *Scorpaena guttata* [13], LC50 for *Poecili reticulata* which is 30.4 mg/L [14], 43 mg/L for *Uca rapax* [15], The 96 hrs LC50 value of cadmium cloride for *Arius arius* was reached to be 56.4 mg/L.[16], 30.06 mg/L of CdCl2 for *Poecilia*

reticulate [17] ,The 50% lethal concentrations (LC50-96 h) of CdCl2 for *Pangasius hypophthalmus* was found at 64.89 and those of CdCl 2 for *C. carpio* was detected at 84.8 mg L [18].

The effect of the metal also depends on the species and size of the fish, salinity of water, water temperature, pH and exposure time. Water temperature ranged in the rearing aquarium between 24-26°C. Temperature is an important factor, which regulates the biogeochemical activities in the aquatic environment such as fish [19], since these values in current study are within the suitable limit for the living and growth for Bunni that leads to increasing food intake, absorption, enzymatic reaction and metabolic activity. The PH of water affects the solubility of many toxic substances and nutritive chemicals; therefore, availability of these substances to aquatic organisms affected [20].

Though the fish survive the initial attack of toxins because of their protective adaptations, the injuries caused by the progressive exposure even in small concentration were manifested at later stages when the organism's resistance weaken due to aging. Also, the condition and response of the test organism to the amount of metal penetrating into its body, the degree of retention and the rate of excretion influence the toxic effect of heavy metal [12].

Generally fish behavior showed abnormalities approximately 1h. after exposure to the various cadmium concentrations such as increase swimming activity, hypersensitivity, fast movements, loss of equilibrium, hyperactivity, hyper-excitability, increase operculum movement, frequent jumping, swimming at the water surface, erratic swimming, spiraling, convulsion, escape attempts from the aquarium and hitting to the walls of the aquarium before finally sinking to the bottom Figure-2. The exposed fishes exhibit tremors and gradual weakening of reflexes leading to imbalance in posture and loss of equilibrium Figure-3. During the experiment a few fishes start drowning by sudden somersaulting Figure-4.



Figure 2- fish sinking to the bottom of the aquarium



Figure 3- imbalance in posture and loss of equilibrium of fish



Figure 4-fish somersaulting in the aquarium.

Erratic movements and abnormal swimming are triggered by deficiency in nervous and muscular coordination which may be due to accumulation of acetylcholine in synaptic and neuromuscular junctions [21].

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 [4].

The decrease in the consumption of oxygen is probably the result of alterations of energy metabolism [22]. Some studies on the pathological effects chemical substances evidenced the gradual destruction of gills filaments, and fish asphyxia [23].

Fish exposed to T1, (0.093 mg/L of cadmium chloride), the gills showed severe to moderate epithelial lifting of secondary lamella and dilation of C.V.S, Figure-5 accompanied with severe to moderate vacuolation of epithelial secondary lamella Figure-6. In T2 (0.093 mg/L) the gills showed moderate epithelial lifting with shortening of secondary lamella and slight cellular infiltration in some secondary lamella Figure-7 with mild secondary lamella fusion, cellular infiltration in secondary lamella and C.V.S. congestion Figure-8.Fish exposed to T3 (0.046 mg/L cadmium chloride) showed protenious homogenous substances and clear shortening of secondary lamella, severe congestion of secondary lamella, great dilation of C.V.S. accompanied with MNCs infiltration Figure-9 Fish exposed to T4 (0.046 mg/L cadmium chloride) showed no clear pathological changes except slight congestion in C.V.S. and primary lamella . The kidney sections at (T1,0.093 mg/L) showed severe necrosis of epithelium with severe degenerative changes and severe depletion of hemopoitic tissues as well as cystic distention of some tubules occur Figure-10. The kidney sections exposed to T2, 0.093 mg/L concentration of cadmium chloride showed slight cellular swelling and reduction in hemopoitic tissues Figure-11, Interstitial congestion with cellular swelling of epithelium lining of renal tubules At T3 (0.046 mg/L) accompanied with severe hemorrhage and blood vessels congestion with heavy mononuclear cellular infiltration in the interstitial tissues mostly plasma cells and lymphocytes Figure-12. The kidney sections At T4 (0.046 mg/L) showed moderate hyperplasia of hemopoitic tissues and slight cellular swelling Figure-13.The specific structural lesions observed in the liver parenchyma exposed to T1 (0.093 mg/L) were moderate to severe necrosis of liver parenchyma with nuclear pyknosis of hepatocyte and congestion of central vein sinusoid Figure-14 At T2, (0.093 mg/L) the fish liver showed moderate cellular swelling of hepatocytes and MNCs infiltration may observed in the lumen of dilated central vein Figure-15 At T3 (0.046 mg/L), the fish liver showed slight necrotic changes in parenchyma, few apoptotic cells, central vein congestion and dilation, moderate dilation of bile duct and MNCs infiltration with slight sinusoidal congestion Figure-16.The Intestine exposed to T1, 0.093 mg/L concentration showed slaughing and necrosis of mucosal epithelium of intestinal villi and diffuse MNCs infiltration Figure-17. At T2 (0.093 mg/L) the intestine showed goblet cell hyperplasia, hypertrophy in the intestinal tissue and diffuse MNCs infiltration consist (macrophage and lymphocyte) Figure-18. At T3 (0.046 mg/L) the fish intestine showed vacuolation of the epithelium in mucosa and severe MNCs infiltration in submucosal layer Figure-19. The specific structural lesions observed in the spleen exposed to T1 (0.093 mg/L) showed severe destruction in spleenic parenchyma, lymphoid depletion in white pulp associated with severe reduction in hemopoitic tissue Figure-20 At T3, (0.046 mg/L), the fish spleen appeared severe blood vessels congestion of red pulp and increase in melanomacrophage clusters Figure-21. At T4 (0.046 mg/L), the fish spleen no pathological lesion was observed except slight congestion in red pulp and spleenic sinuses.

The gill histopathological finding in the present study is similar to the observation by [24] that the basic gill pathology, hyperplasia, detected in the majority of studied fish, and mild to sever 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) indicated the secondary defense mechanism of the body [25, 26].The blood vessels near the injury side dilate and permeability of capillary which produced exudate of fluids and resulted in congestion and blood derived exudate can inter the adjacent epithelium, lamellar anurysim occurred by rupture of pillar cells which allowed expansive vascular space predispose to develop hemorrhage [27]. The liver histopathological finding in the present

study confirms and is similar with the observation by [28] that refered to the basic types of liver histopathological changes in gibel carp include vacuolisation of hepatocytes, karyopyknosis, and necrosis of hepatocytes. Liver is one of the secondary site of cadmium accumulation, and the first site of detoxification [13, 29, 30]. 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 [31]. 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.

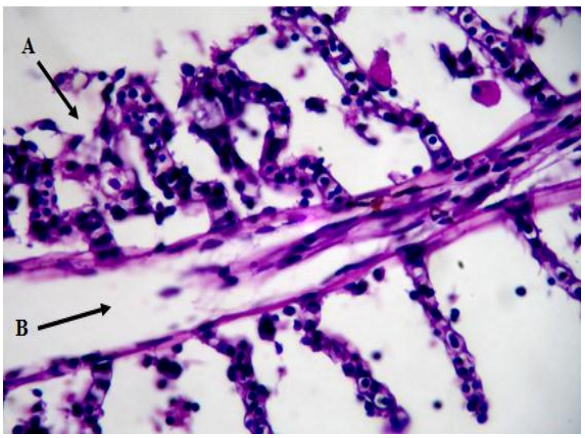


Figure 5- Gills section of T1 group shows severe to moderate epithelial lifting of secondary lamella (A) and dilation of C.V.S (B) (H&Ex40).

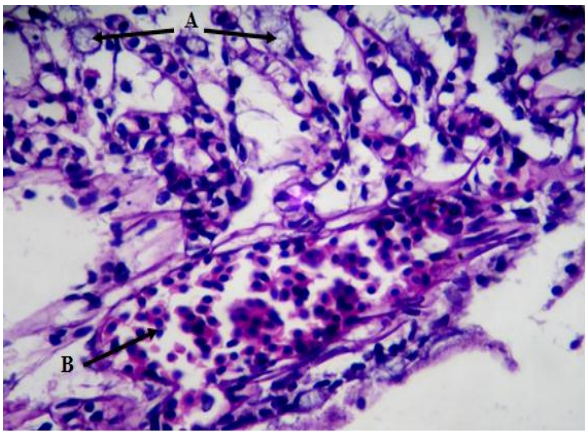


Figure 6- Gills section of T1 group shows severe to moderate vacuolation of epithelial secondary (A) with great dilation and congestion of C.V.S (B) (H&EX40)

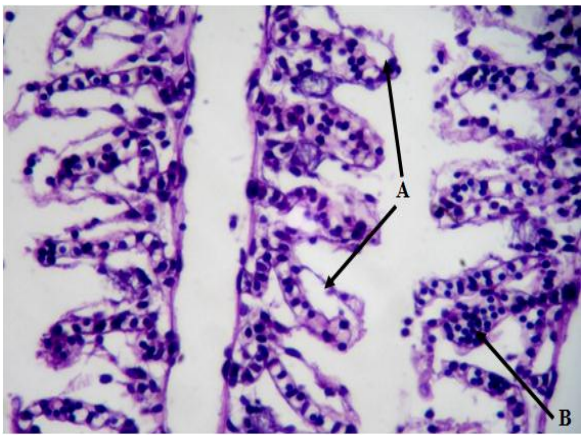


Figure 7- Gills section of T2 group shows moderate epithelial lifting with shortening of secondary lamella (A) and slight cellular infiltration in some secondary lamella (B) (H&Ex40).

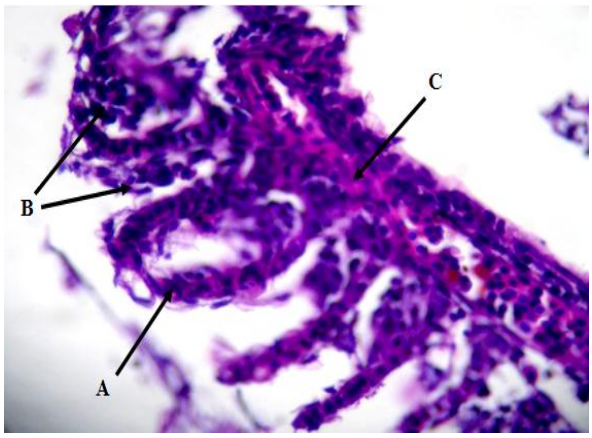


Figure 8- Gills section of T2 group shows mild secondary lamella fusion (A) cellular infiltration in secondary lamella (B) and C.V.S. congestion(C) (H&Ex40).

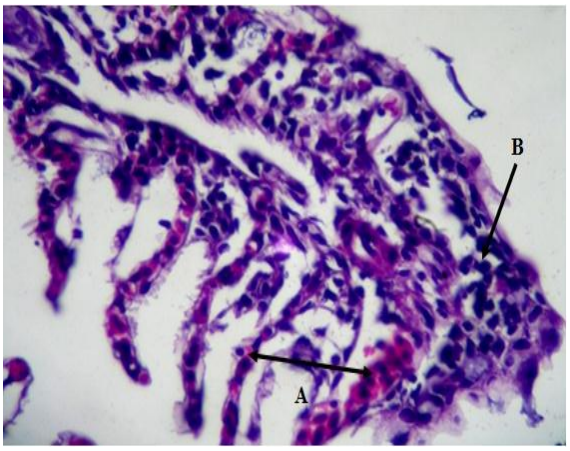


Figure 9- Gills section of T3 group shows severe congestion of secondary lamella, great dilation of C.V.S. (A) accompanied with MNCs infiltration (B) (H&Ex40).

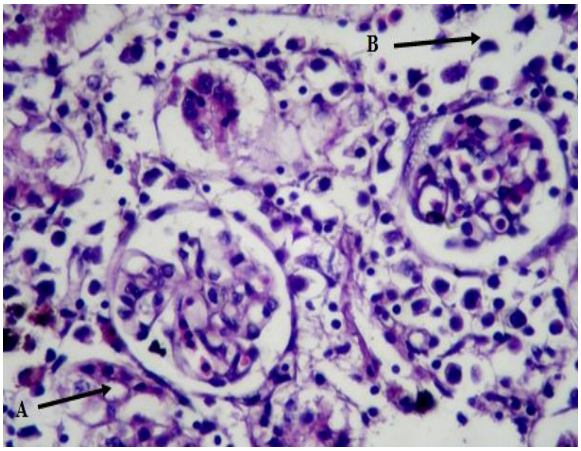


Figure 10- kidney section of T1 group shows severe degenerative changes with necrosis of renal tubules (A) and severe depletion of hemopoitic tissues (B) (H&Ex40).

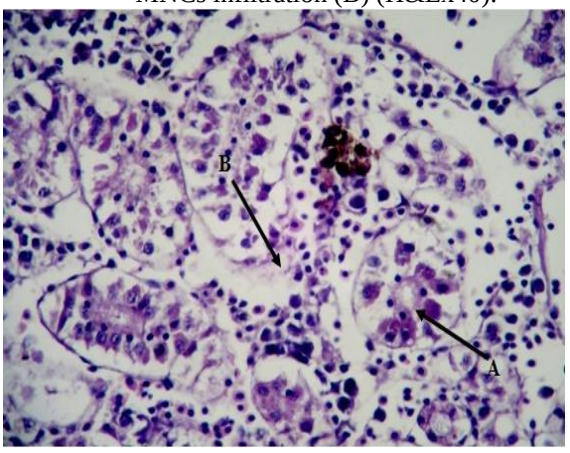


Figure 11- kidney section of T2 group shows cellular swelling (A) and reduction in hemopoitic tissues (B) (H&Ex40)

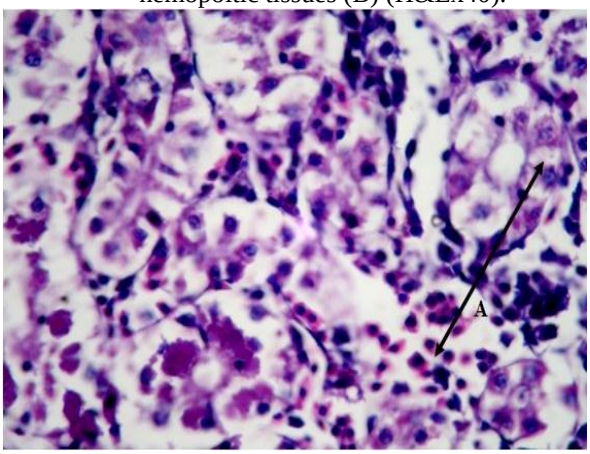


Figure 12- kidney section of T3 group shows interstitial hemorrhage with cellular swelling of epithelium lining of renal tubules (A) (H&Ex40).

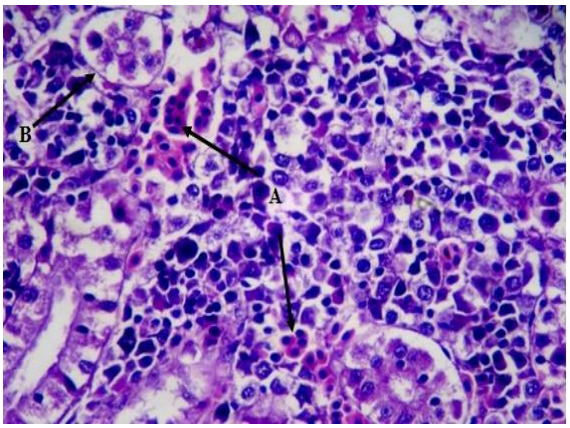


Figure 13- kidney section of T4 group shows moderate hyperplasia of hemopoitic tissues (A) and slight cellular swelling (B) (H&Ex40).

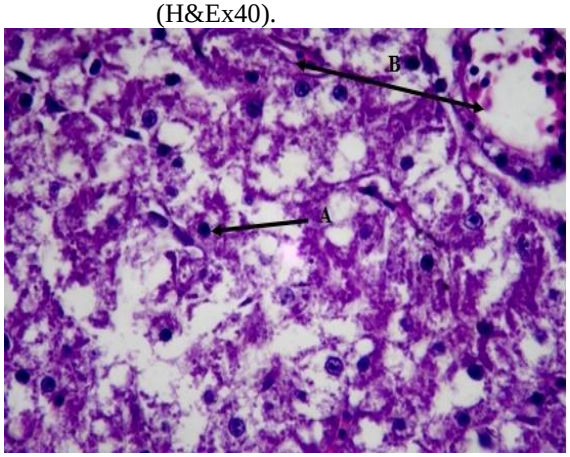


Figure 14- liver section of T1 group shows moderate to severe necrosis of liver parenchyma with nuclear pyknosis of hepatocyte (A) and congestion of central vein sinusoid (B) (H&Ex40).

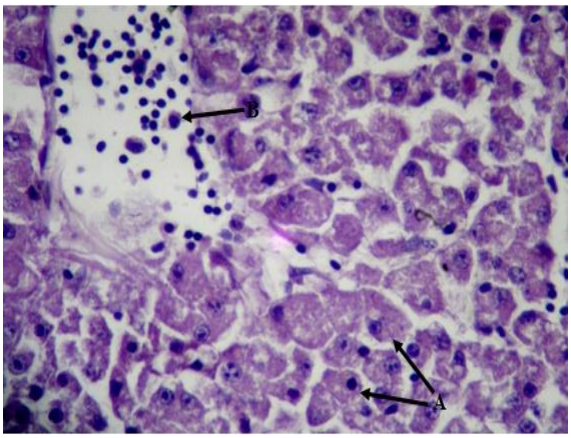


Figure 15- liver section of T2 group shows acute cellular swelling of hepatocyte (A) and MNCs infiltration observed in the lumen of dilated central vein (B) (H&Ex40).

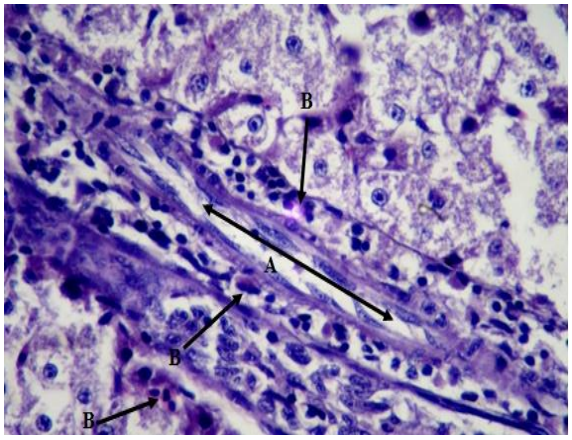


Figure 16- liver section of T3 group shows moderate dilation of bile duct (A) and MNCs infiltration with slight sinusoidal congestion(B) (H&Ex40).

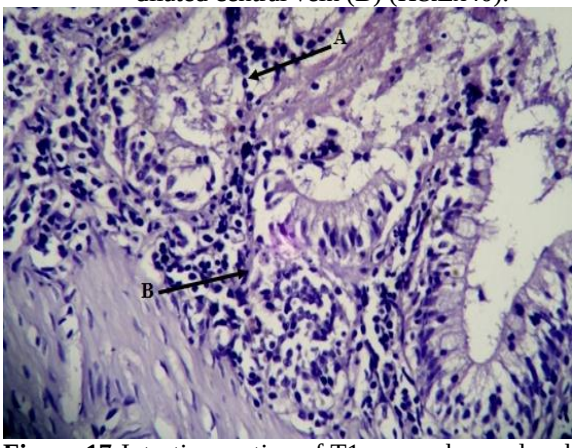


Figure 17-Intestine section of T1 group shows slaughing and necrosis of mucosal epithelial of intestinal villi (A) and diffuse MNCs infiltration(B) (H&Ex40).

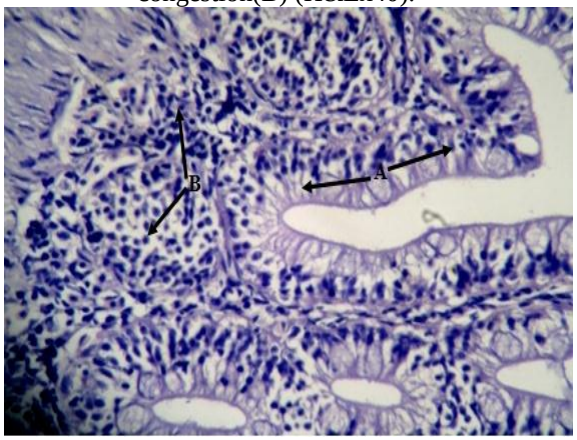


Figure 18- Intestine section of T2 group shows goblet cells hyperplasia and hypertrophy in the intestinal tissue (A) and sub mucosal diffuse MNCs infiltration consist of macrophages and lymphocytes (B) (H&Ex40).

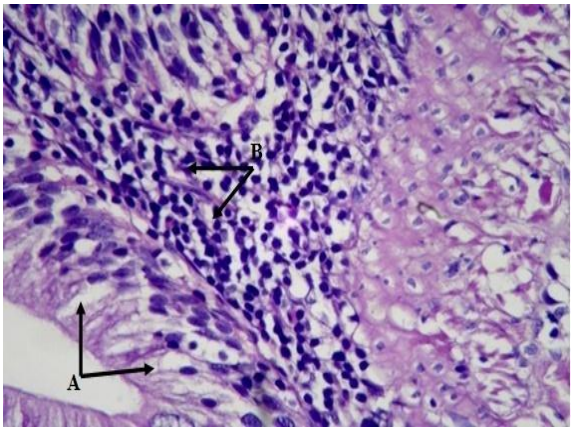


Figure 19- Intestine section of T3 group shows vacuolation of intestinal epithelium in mucosa (A) and severe MNCs infiltration in submucosal layer (B) (H&Ex40).

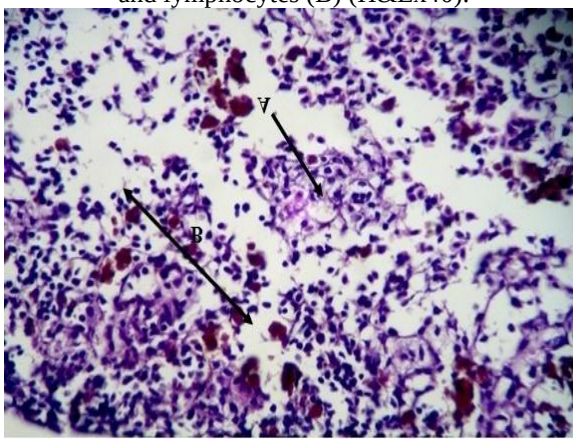


Figure 20- Spleen section of T1 group shows severe destruction in spleenic parenchyma, lymphoid depletion in white pulp (A) associated with severe reduction in hemopoitic tissue(B) (H&Ex40).

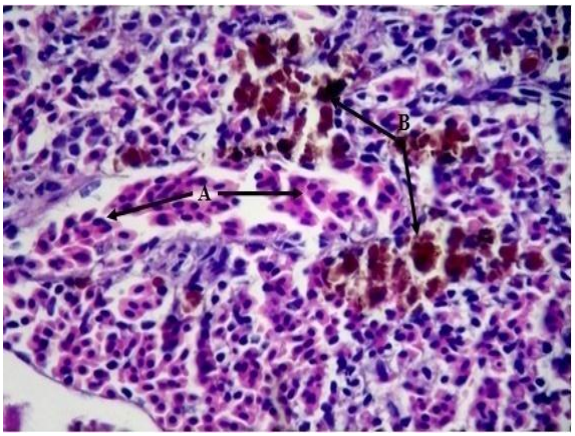


Figure 21- Spleen sections of T3 group shows severe blood vessels congestion of red pulp (A) and increase in melanomacrophage cluster (B) (H&Ex40).

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