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Study of Physiological and Histological Effects Under very low Concentration of Cyanobacterial toxin MC-LR on Lab. Mice (*Mus musculus*).

Emad YA Al-Sultan*, Majda I Abd Al Majeed, and Abbas AK Abbas.

Department of Biology, College of Education for Pure Science, Basra University, Iraq.

ABSTRACT

Microcystin is cyanobacterial toxin (Hepatotoxin) and the lethal dose concentration reach to 50 µg / kg/ day , study included using very low concentration of cyanotoxin microcystin-LR (first dose 0.05 and the second dose 0.14 µg / kg/ day) for two periods at acute injection (after 48 h) and chronic (after two week) to evaluate certain physiological effects and histopathological changes in laboratory mice after i.p injection. Hemoglobin and packed cell volume was decreased significantly (P≤0.05) with increase doses and periods of injection compared with control group. Liver enzymes (AST, ALT, and ALP) increased significantly in particular at second dose receiving group as chronic injection reached (70.25, 169.75 and 276 IU/L, respectively) and followed by first dose receiving group of MC-LR compared with control group. Many histopathological changes were obvious in liver and kidney of laboratory mice was increased with increasing of doses and periods of injection. In liver at acute injection necrosis of hepatocyte as pyknosis of nuclei , karyorrhexis of necrotic hepatocytes nuclei with pyknotic nuclei where showed for two doses , while chronic injection showed hydropic degeneration with hypertrophy of hepatocytes , pyknosis of necrotic hepatocyte nuclei , infiltaration of lymphocytes , karyolysis, hydrobic degeneration for two doses . kidney under first and second doses in acute injection showed some histopathological changes represented by shrinkage glumerules , dilation of bowman capsule space , necrosis of cell blood capillaries and some renal tubules , while at chronic injection under second dose several changes were showed represented by hypertrophy, hyperplasia with disappearance of renal tubules cavity or its narrowed, hypertrophy of sequamous epithelial cells of glomeriular arterioles , metaplasia of sequamous epithelial tissue of bowman capsule wall in to cuboidal epithelial tissue .

Keywords: Cyanobacterial toxins (MC-LR) ,Liver , Kidney , Physiological and histologiacl effects

Corresponding author

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INTRODUCTION

Blue-green algae produce a variety of toxins, subsequently called cyanotoxins , that are classified functionally into hepato- , neuro- and cytotoxins. Additionally cyanobacteria produce lipopolysaccharides (LBS) as well as secondary metabolites that are potentially pharmacologically useful. Cyanotoxins divided into three groups: cyclic peptides (the hepatotoxins microcystins and nodularins), alkaloids (the neurotoxins, anatoxins and saxitoxin) and Lipopolysaccharides (Bettina *et al*., 2000) . The most algal species often implicated with toxicity are *Microcystis aeruginosa*, *M. flos-aquae*, *Plantothrix* (= *Oscillatoria rubescence*) , *Aphanizomenon flos-aquae*, *Anabaena flos-aquae , Plantothrix agardhii , Nostoc muscurum , Anabaena variabilis , Hapalosiphon welwitschii , Calothrix parietina and Lyngbya spp.* (Bettina *et al*., 2000; AL-Sultan , 2007; AL-Sultan and Al-Aarajy, 2008) .

Cyanobacteria, also known as blue-green algae, are a family of single-celled algae that proliferate in water bodies such as ponds, lakes, reservoirs, and slow-moving streams when the water is warm and nutrients are available (Butler *et al*., 2009). Toxic microcystins are actively absorbed by fish, birds and mammals. Microcystin primarily affects the liver, causing minor to widespread damage, depending on the amount of toxin absorbed. People swimming, waterskiing, or boating in contaminated water can be exposed to microcytins. Microcystins may also accumulate in fish that are caught and eaten by people. Finally, pets and livestock have died after drinking water contaminated with microcystins. (Izaguirre *et al*., 2007).

Microcystins are cyclic peptides which are small molecules with a molecular weight ranging from 800-1000 Dalton (Botes *et al*., 1985). The liver is the major target organ of microcystin toxicity as it was shown to accumulate 20-70 % of radioactivity labeled toxin dose intravenous. Studies in mice and pigs exposed to extracts at a toxic *Microcystis aeruginosa* bloom demonstrated dose dependent toxicity (Falconer *et al*., 1999).

Microcystins are produced by the cyanobacterial cells. When the algae die, the cell walls burst, releasing the toxin into the water. Microcystins are extremely stable and resist common chemical breakdown such as hydrolysis or oxidation under conditions found in most natural water bodies. These toxins can break down slowly at high temperature (40C° or 104 CF) at either very low pH (<1) or high (>9) (Harada, 1996). The half-life of the toxin to degrade at pH=1 and 40 C° is3 weeks at typical ambient conditions half-life is 10 weeks. Microcystins break down slowly in full sunlight especially when water soluble pigments are present (Tsuji, K., *et al*., 1995).

The aim of this study to evaluation toxic effects of very low concentrations of hepatotoxins (MC-LR) under the acceptable concentration in the environments according to WHO (1 µg /L) onliver and kidney of laboratory mice ((*Mus musculus*) .

MATERIAL AND METHODS

Animals

Adult male 12-weeks old albino mice (*Mus musculus* L.) weighing 25-30g were used in the present study. They were housed and maintained in controlled condition animal house (25±2C°) and 50% relative humidity with 12:12 light- dark cycle. Food and water were given orally.

Chemicals

Cyanotoxin (Microcystin-LR) were purchased from Alexis company (USA) to prepare the different concentrations (0.05 µg/kg and 0.14 µg/kg) , The toxin doses dissolved in normal saline .The kits for estimation of aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) were purchased from biondox, Biomerieux were used to calculate enzyme concentration in blood of mice.

Treatments

Forty mice were equally divided into five experimental groups. They were treated as follows: Group (1) received normal saline (0.1 ml /kg/day) as control group. Group (2) received 0.05µg/kg/day of cyanotoxin (MC-LR) for 48 h as acute exposure. group (3) received 0.05µg/kg/day for two week (14 days) as chronic

exposure . Group (4) received low dose of MC-LR 0.14µg/kg/day for 48h as acute exposure, and group (5) received 0.14µg/ kg/day for two week as chronic exposure . All treatment were given in 0.1 ml intraperitoneal injection.

Biochemical parameters and measurements

After 48h the mice of group 2 and group 4, were scarified by cervical dislocation under ether anesthesia and venous blood samples were collected by direct heart puncture. The same procedure was performed after two week for the group 3 and 5. The blood samples were divided into two portions, one was collected in clean tubes with few drops of EDTA for complete blood parameters, and the other was centrifuged, serum was recovered, and diagnostic kits were used to determine the activity of AST, ALT, and ALP enzymes.

Histopathological Examination

Tissue specimens from liver and kidneys were obtained from each group and fixed in 10% formalin and embedded in paraffin. After routine processing , paraffin sections were cut into 5µm thickness and stained with haematoxylin and eosin (Humanson, 1972).

Statistical analysis

The results were analyzed using SPSS program version -17. One way ANOVA and R.LS.D were done to compare between means of treatments

RESULTS AND DISCUSSION

Biochemical and physiological parameters

The results showed significant decrease ($p \leq 0.05$) of hemoglobin after acute and chronic exposure to second dose of MC-LR reach to 9.75 g/L and 10.75 g/L respectively . Similarly, the first low dose of this toxin caused a reduction in hemoglobin that in comparison with control group.. Pocket cell volume decreased significantly after exposure to the high level (second dose) after acute and chronic injections, which reach to 29.25 g/L and 33g/L, respectively. Likewise, the low concentration of toxin caused a considerable reduction in PCV value that in comparison with control group for both periods (**table-1)**. this effects may be return to the ability of microcystins to analyze the membranes of the RBCs especially at high concentration as a result to conjugate between microcystins and free radicals of protein (SH) and disrupted cell membrane permeability within few minutes (Grabow *et al*., 1982 ; Sicinsk *et al*., 2005) ,in addition this results agree with study of AL-Sultan *et al*., (2011) distinguished by decrease in mean of RBCs, WBCs, HB, and PCV in blood plasma of juvenile fish *Ctenopharyngodon idella* when fed on toxic blue-green alga *Nostoc muscurum* .

The enzyme AST increase significantly only at highly toxin injection after 48 h reach to 70.25 IU/ L but led to non significant increasing in other treatment compared with control group , while ALT enzyme showed significant increasing under high level of toxin after 48 h reach to 169.75 IU/L flowed by 135 IU/L after two week of low level injection compared with control group, Also ALP enzyme showed highly significant increasing reach to 276 IU/L at acute exposure and after chronic exposure 211.75 IU/L of second dose in addition this enzyme increasing significantly after expose to low toxin for two period compared with control group **table-1.** This increasing of activity of enzymes may be return to the toxic effect of MC-LR which induction alkaline phosphatase activity only in the liver these increases indicate that the membrane properties are perturbed by interaction with MC-LR variants and these enzymes are intrinsic plasma membrane enzymes involved in membrane transport activities and in bone formation (Mazorra *et al*., 2002), so this results showed the low concentration of MC-LR under maximum acceptable concentration also causes similar effects with high doses of this enzymes .

Histopathological effects

Liver

First dose (0.05 µg/kg/day)

After 48hr of toxin injection some histopathological effects in liver was showed which included the beginning necrosis of hepatocytes as pyknosis of nuclei. These pyknotic nuclei are characterized by shrinking , dark and condensing of chromatin (fig.-2). While after two week of injection , the hydropic degeneration with hypertrophy occurred in some hepatocytes in addition to pyknosis of necrotic hepatocytes nuclei compared with control group (fig- 1,3).

Second dose (0.14 µg/kg/day)

At high dose after 48hrs of injection several histopathological effects were showed included karyorrhexis of necrotic hepatocytes nuclei in several sites of liver tissue with pyknotic nuclei (fig-4). But in chronic injection after two week local infiltration of lymphocytes and karyorrhexis or karyolysis of necrotic hepatocytes nuclei were noticed in addition to hydropic degeneration of other hepatocytes compared with control group (fig- 5). the toxic effects on liver may be return to effects of microcystins on hepatocytes , where they inhibit

protein phosphatase (pp) by covalently binding the enzyme in the case of microcystins . this binding blocks proteins dephosphoration , affecting cytoskeleton proteins too. And additional target of microcystins is β-sibunit of ATP synthase ,causing mitochondrial apoptotic signaling (Valtere *et al*., 2008).

Kidney

First dose (0.05 µg/kg/day)

In acute and chronic injection after two week of treatment, the histopathological effects included the shrinkage of some glomuruli, dilation of bowman capsule space, necrosis of cells of blood capillaries wall of the glomerulus (glomerular arterioles) and necrosis some of cells of renal tubules compared with control group (fig-7).

Second dose (0.14 µg / kg/day)

After acute injection (48hr), kidney was characterized by fibreniod exudates in renal tubules cavity (fig-8), also hyperplasia is starting in epithelial cells of renal tubules walls (fig-9). While in chronic injection after two week was led to histopathological changes which included hypertrophy of renal tubules cell walls and hyperplesia , disappearance of renal tubules cavity or its narrowing (fig-10) and the hypertrophy of sequamous epithelial cells of glomerular arterioles and metaplasia of squamous epithelial tissue of bowman capsule wall (parietal wall) into cuboidal epithelial tissue (fig.-11). these results agree with study of John *et al*., (2014) which their showed some histopathological changes in kidney of swiss mice exposed to high doses reach to 625 and 1.250 mg/kg of cyanobacterial extracts, Also these results agree with study of Gupta and Guha (2006) and AL-Ali *et al*., (2011), while Palikova *et al*., (2004) mentioned no changes were found in the kidney of *C. carpio* when exposure to cyanobacterial extracts and also disagree with results of Carvaleho *et al* ., (2007) when showed expose low doses of cyanotoxins (1.77 to 6.12 µg L^{-1} total microcystins) in mouse drinking test solutions did not result in histological injury in the kidney of animals exposed to cyanotoxins , while Rabergh *et al*., (1991) observed in kidney of common carp *Cyprinus carpio* L. the bowmans capsules of glumeruli were dilated and degeneration when fish injected with a lethal dose 550 µg / kg of microcystin -LR.

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Table 1: Some physiological parameters in blood of mice after acute and chronic exposure to cyanotoxins (Microcystins-LR)

Physiological parameter	control	First dose 0.05 ug/kg/ day after		Second dose 0.14 ug/kg/day after		R.L.S.D
		48 h	14 days	48h	14 days	
HP mg/l	14.12 ^{a}	11.25 ^{b}	12^{b}	9.75^{c}	10.75^{c}	1.15
PCV mg/l	42.37^{a}	33.75 ^{c}	36^{b}	29.25^{d}	33^{c}	3.34
AST IU/l	40.25 ^{b}	48 ^{b}	43^{b}	70.25^{a}	47.75^{b}	13.75
ALT IU/l	97 ^{c}	90 ^{c}	135^{b}	169.75^{a}	88.75^{c}	30.09
ALP IU/l	142.5 ^{d}	157^{c}	175.25^{c}	276^{a}	211.75^{b}	22.58

Note : similar letter = significant difference P≤ 0.05 Different letter= non-significant differences

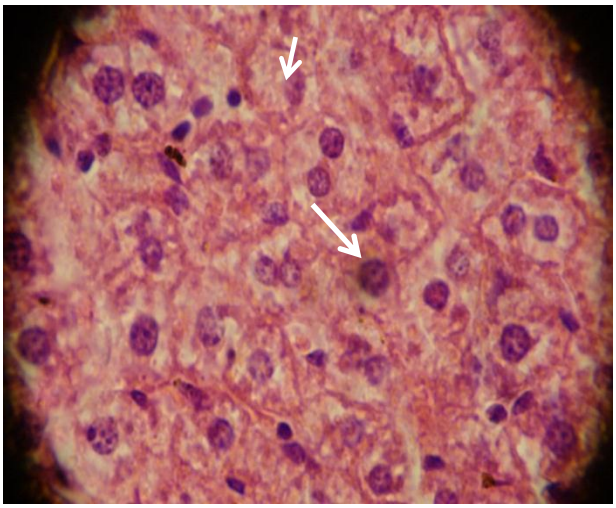

Fig-2: Liver image treated with first dose of MC-LR (0.05 µg/kg/day) after 48h (acute exposure) showed pyknosis of nuclei (arrow) 400X .

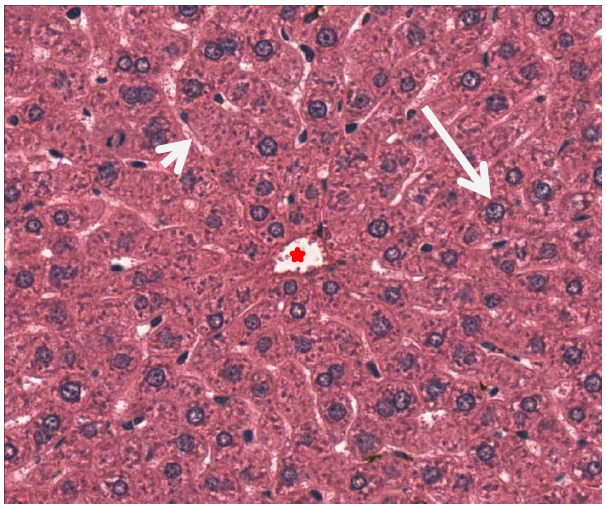

Fig-1: Liver Control image showed central vein (star) Hepatocyte (long arrow) and sinusoids (short arrow) 400 X .

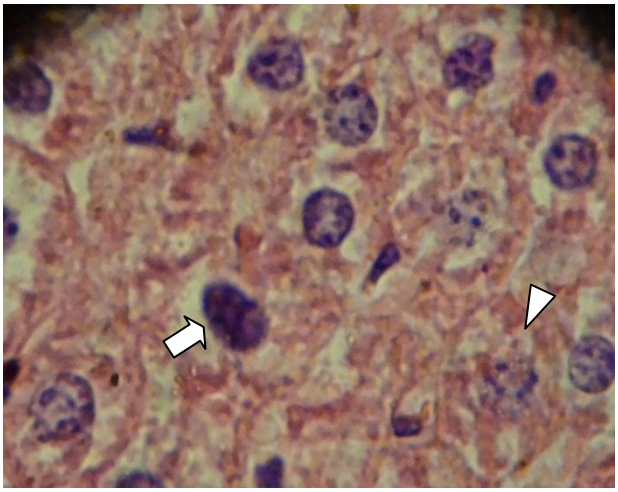

Fig-3: Liver image treated with first dose MC-LR (0.05 µg/kg/day) after two week showed hydrobic degeneration (head arrow) and pyknosis of necrotic hepatocytes nuclei (arrow) 400X .

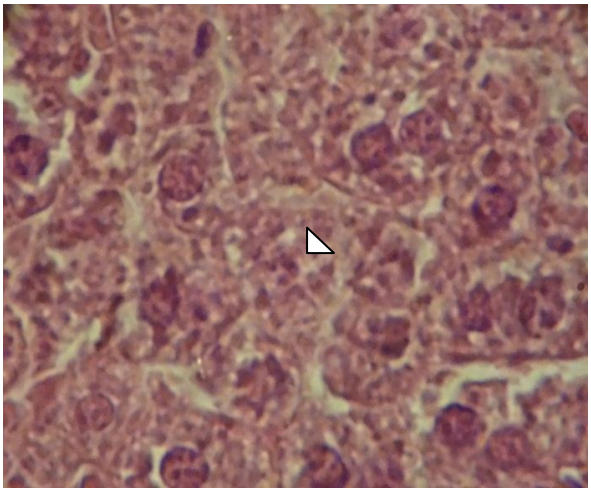

Fig-4: Liver image treated with second dose MC-LR (0.14 µg/kg/day) after 48h showed karyorrhexis of necrotic hepatocytes nuclei (-head arrow) 400X .

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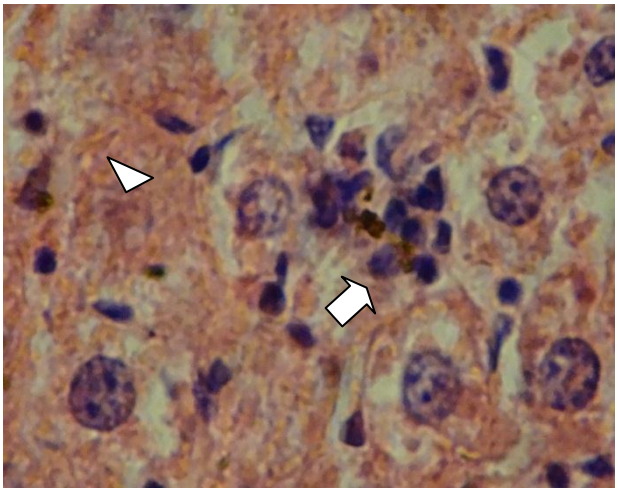


Fig-5: Liver image treated with second dose MC-LR (0.14 µg/kg/day) after two week showed infiltration of lymphocytes (arrow) and karyolysis of necrotic hepatocyte nuclei (head arrow) 400X .

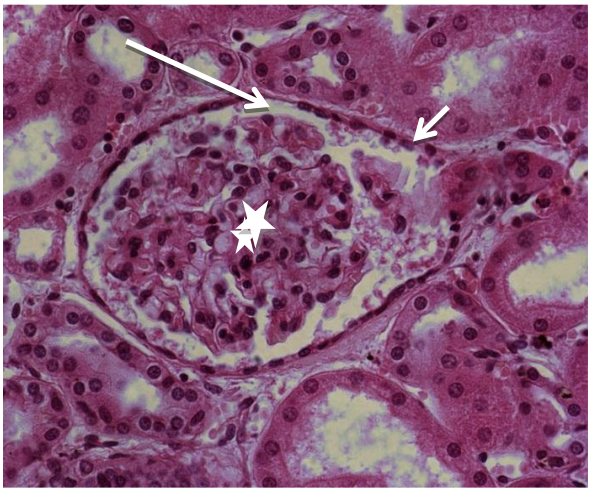


Fig-6: Kidney image of control mouse showed bowman space (long arrow), glomerulus (stars), parietal wall of browman capsule (short arrow) and renal tubules (double star) 400X

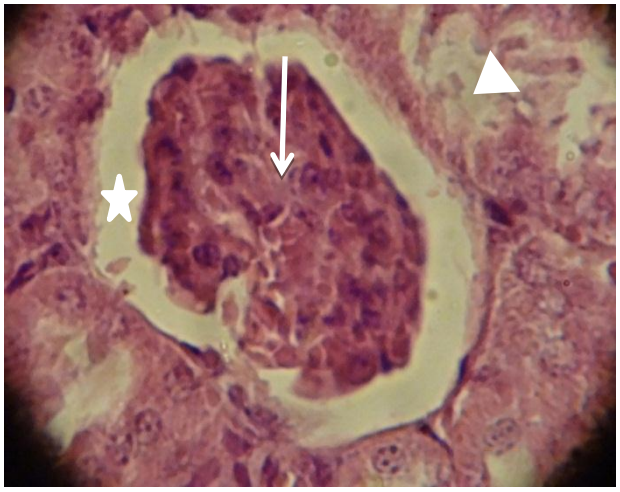
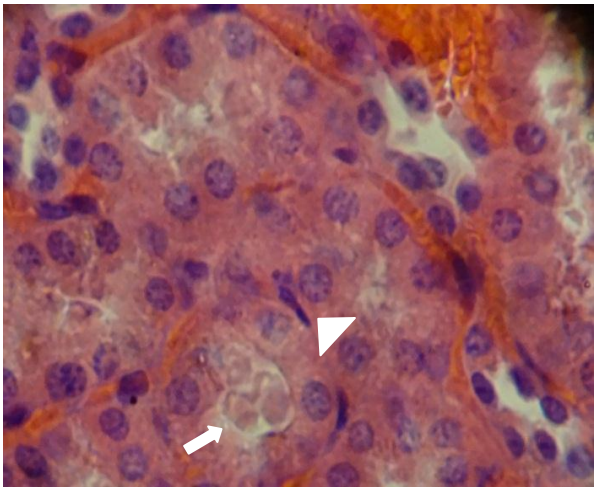


Fig-7: Kidney image treated with first dose MC-LR (0.05 µg/kg/day) after 48 hr showed some shrinkage of glomerulus (arrow), dilation of bowman capsule space (star) and necrosis of some renal tubules cells (head arrow) 400X .



- Fig-8: Kidney image treated with second dose MC-LR (0.14 µg/kg/day) after 48h showed hyperplasia in epithelial cells of renal tubules walls (head arrow) and fibroid exudates in renal tubules- (arrow) 400X.

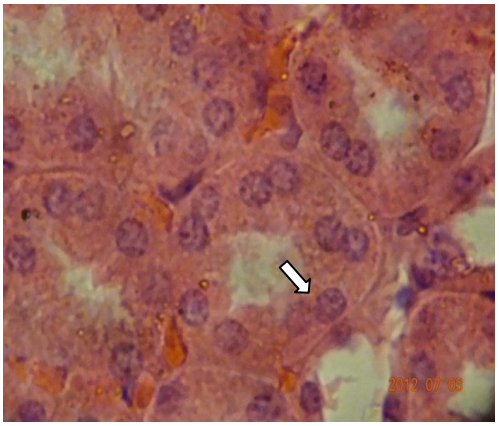


Fig-9:Kidney image treated with second dose MC-LR (0.14 µg/kg/kg) after 48h showed hyperplasia in epithelial cells of renal tubules walls (arrow) 400X .

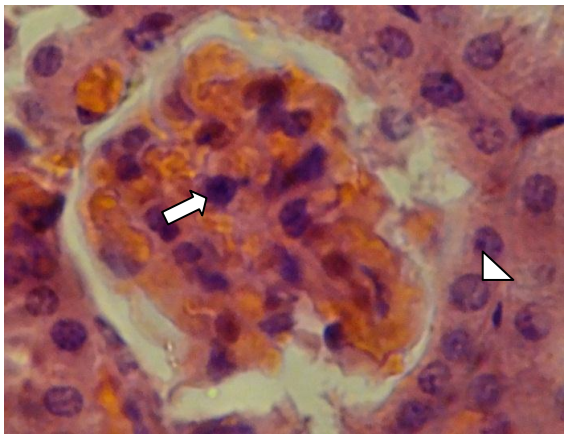


Fig-10: kidney image treated with seconddose MC-LR (0.14 µg/kg/day) after two week showed hyperatrophy of squamous epithelial cells of glomular arterioles (arrow) and metaplasia of squamous epithelial tissue of bowman capsule wall (parietal wall) in to cuboidal epithelial tissue (head arrow) 400X

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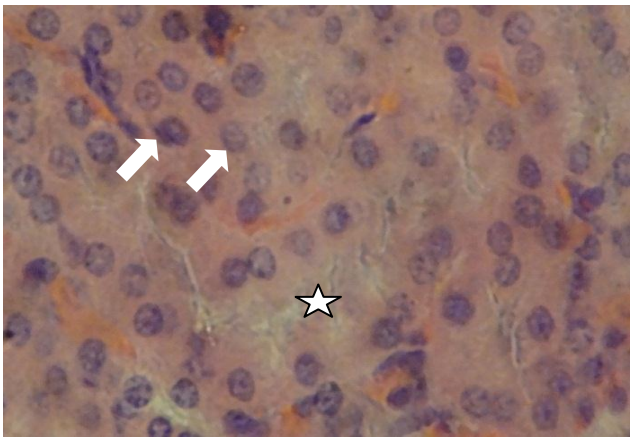

Fig-11: kidney image treated with second dose MC-LR (0.14 µg/kg/day) after two week showed hyperatrophy of renal tubules cell walls and hyperplasia (arrow) disappearance of renal tubules cavity and became narrow (star) 400X .

CONCLUSION

Present study revealed that the low concentrations of cyanotoxins MC-LR (0.05 and 0.14 µg/kg) less than acceptable concentration of World Health Organization (WHO) which producing from cyanobacteria (Blue –green algae) which have physiological and histological effects satisfactory mice laboratory and especially the liver and kidney in acute and chronic injections and increasing effects with increasing of period injections are similar to the effects of high concentrations, giving evidence of the seriousness of the toxins the liver on living organisms .

Recommendation

The present study recommends the importance of attention to toxins produced from algae greens blue-especially toxins hepatic a tailored after a study that showed concentrations few of which cause the effects of toxicity in the blood of laboratory mice and liver enzymes and damage tissue to the liver and kidney, so to be from attention, drinking water, some food and a statement they are free of those toxins to avoid the harmful effects .

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