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# Hepatotoxicity of Microcystin-LR in Wistar Rats

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## Abstract

Microcystins produced by cyanobacteria have been identified as potent hepato and neurotoxicants to human and livestock. The present study was aimed to determine the possible hepatotoxic effects of MC-LR on mammals using male Wistar rats as an animal model. Thirty-five rats were divided into five groups (n=7 in each). Test groups were orally treated with different doses of MC-LR (0.105 µg/kg, 0.070 µg/kg and 0.035 µg/kg). Well water contaminated with MC-LR (0.091 µg/kg) was given to the environmental exposure group and distilled water was administered to the control group. Body weight was measured once a week. The total duration of exposure of the rats was 90 days. Blood samples were collected at 0, 7, 14, 28, 42, 60, 90 days. Serum concentrations of Aspartate Amino Transferase (AST) and Aspartate Alanine Transferase (ALT) were analyzed from each blood sample. At the end of the experimental period, liver samples were collected for histological examination following accepted protocols. The mean bodyweight of the rats in treated groups of rats gradually increased until the twelfth week and decreased thereafter. A significant decrease in body weight of rats with MC-LR exposure was seen at 13 and 14 weeks, compared to the control group ($p=0.000$). The absolute and relative weights of livers of the treated groups were significantly lower than the control group ($p<0.05$). The highest serum AST and ALT levels were observed in rats that were given MC-LR at the dose of 0.105 µg/kg. The hepatocytes showed mild changes including sinusoidal congestion and vascular congestion with lobular inflammation, focal hemorrhages and marked microvesicular steatosis in 0.105 µg/kg group. Mild lobular inflammation, focal hemorrhage, perivenular inflammation, prominent Kupffer cells and focal microvesicular steatosis were observed in 0.091 µg/kg group. Mild lobular inflammation was seen in the group given the dose of 0.070 µg/kg. In conclusion, this study demonstrated that long-term exposure to MC-LR can cause hepatotoxicity in Wistar rats.

**Keywords: Microcystin-LR (MC-LR); Wistar Rats; AST; ALT; Histopathology**

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## Introduction

Microcystins (MCs) are the most common toxins produced by cyanobacteria. These poisons can increase in concentrations that are harmful to human health during cyanobacterial "blooms" (mass development) in freshwater ecosystems. Toxic cyanobacteria poisoning outbreaks in animals and people have been documented in a number of countries [1]. Exposure to MC can cause liver failure and mortality in humans and a variety of animals, making it a major public health problem [2].

Microcystins (MC) are generated by *Planktothrix*, *Microcystis*, *Aphanizomenon*, *Nostoc*, and *Anabaena* species [3]. The general structure of Microcystin (MC) is cyclo-(D-Ala1-X2-D-MeAsp3-Z4-Adda5-D-Glu6-Mdha7), where X and Z are variable L-amino acids, D-MeAsp represents D-erythro-β-methylaspartic acid, Adda the unusual amino acid (2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid and Mdha N-methyldehydroalanine [3] (Figure 1).

Microcystins include more than 80 structural variations of cyclic heptapeptides [5]. The lipophilicities and polarity of different MC variants affect their toxicity [6]. The chemical "shape" of the Adda-glutamate component of microcystins in solution is comparable to that of nodularin's, another category of cyanobacterial hepatotoxins [7,8]. Recent research has revealed that the Adda region is critical for protein phosphatase protein molecule interactions, making this area critical for cyanotoxin toxicity [9,10].

However, exceeding this level of MC can be tolerated, if elimination or discontinuation of exposure is expected in the near future. A small number of epidemiological research suggest that MC exposure may be one of the factors linked to a high incidence of primary liver cancer. These studies include one from China, where people drank cyanobacteria-contaminated pond-ditch water [3,11], and another from central Serbia, where cyanobacteria-contaminated water was used as a source of drinking water [3,11]). However, it has been proposed that the presence of MC in drinking water can be linked to the development of primary liver cancer only when additional variables such as aflatoxin and alcohol are present [12-14].

Considering the toxic nature and the influence of toxicity on human health the World Health Organization (WHO) established a provisional guideline limit of 1 µg/L for MC-LR [15]. Further Tolerable Daily Intake (TDI) for humans was established as 0.04 µg/kg of body weight/day for MC-LR [16].

Despite the fact that the liver is the major organ in which MC induces tissue fibrosis and irreversible damage to hepatic cells [17]. The liver is the major organ affected by MC-LR toxicity, which results in cytoskeletal disruption, necrosis, blood pooling, weight gain, and hepatocyte blabbing. Hemorrhagic shock can sometimes cause liver damage in rats and other lower vertebrates [18]. In the detoxification of Microcystins, the liver plays a crucial role [19]. This is accomplished through three mechanisms: (1) glutathione S-transferase conjugation, (2) cysteine conjugation, and (3) oxidized ADDA diene conjugation [20]. The glutathione peroxidase pathway plays an important role in recovery from MC intoxication. All three conjugates are actively eliminated through the biliary route [21] and can be identified in urine, feces, and liver cytosolic fractions [19]. MC-LR conjugates of glutathione and cysteine are less hazardous than parent MC-LR.

It's possible that the low toxicity of MC-LR conjugates is due to an imbalance between accumulation and effective removal [22]. Because MC-LR is unable to pass cell membranes, it does not enter cells in most tissues. MC-LR is primarily absorbed through the mouth. Although skin absorption is improbable, inhalation is a viable option. Absorption occurs mostly in the small intestine and to a lesser extent in the stomach [23]. The majority of MC-LR absorbed from the gastrointestinal tract (78% to 88%) is resorbed by the portal blood stream to the liver, where it penetrates hepatocytes *via* a multi-specific bile acid transport mechanism.

There have been more reports recently about the frequency and intensity of blooms in reservoirs in Sri Lanka's North Central, North East, and Uva provinces [24-26]. Most importantly, scientific research has revealed that toxin-producing cyanobacterium *M. aeruginosa*, *Cylindrospermopsis* sp., and *Anabaena* sp. have contaminated the majority of drinking water reservoirs, with analytical results demonstrating a significant correlation between cyanobacterial cell density and cyanotoxins in drinking water sources [26-29].

As a result, the current study used male Wistar rats as an animal model to assess the effects of sub-chronic exposure to purified different doses of MC-LR and MC-LR contaminated water that align with the WHO recommended standard concentration of MC-LR for drinking water on the liver via oral gavage over a period of time.

## Methods

The ethical clearance was granted by the Ethics Review Committee of the Faculty of Medical Sciences (An.E.17/18), University of Sri Jayewardenepura. The study was carried out in accordance with the Ethics Review Committee's relevant guidelines and regulations.

### Animals

Male Wistar Rats (183.2 ± 0.24 g) were acclimatized for one week at the Animal House, Faculty of Medical Sciences, University of Sri Jayewardenepura, following purchase from the Medical Research Institute (MRI) of Sri Lanka. Unless otherwise stated, rats were fed and given water ad libitum and kept on a 12 h light/dark cycle at 28°C ± 2°C. Water and food consumption were measured, and animal behavior was observed throughout the study.

### Experimental design

Three doses of MC-LR were prepared in this study based on WHO standard recommendations for MC-LR in drinking water: Higher than the WHO standard (HW) 1.5 µg/L, equal to the WHO standard (W) 1 µg/L, and lower than the WHO standard (LW) 0.5 µg/L. According to the mean initial body weights of the rats fed with the above concentrations, the pure MC-LR was HW=0.105 µg/kg, W=0.070 µg/kg, and LW=0.035 µg/kg. Well water collected from the Padaviya area of Sri Lanka's North Central Province contained 1.3 µg/L, which was higher than the WHO recommended level. Accordingly, Environmental exposure group (EN) received (0.091 µg/kg) of MC-LR. The control group was given distilled water. For 90 days, the solutions, well water, and distilled water were fed. The mouse groups were labeled as HW, W, LW, EN, and control based on the solutions they received. Throughout the experiment, rats' behavior, as well as their water and food consumption, were recorded every two days. To avoid contamination of urine samples with food dust, nutritionally complete solid food was provided ad libitum. Prior to administration, the MC-LR solutions were formulated biweekly and adjusted to reflect weight changes in the treated groups. After 90 days of dosing, the animals were euthanized, weighed (g), and the livers were resected out with minimal trauma.

### Collection of blood

For clinical chemistry, venous blood samples were drawn from the lateral tail vein at 0, 7, 14, 28, 42, and 60 days. At the end of the experiment (90 days), blood was collected *via* cardiac puncture while the rats were anesthetized prior to euthanasia for both hematological and chemical analysis. To prevent coagulation, blood for hematological analysis was transferred to a 1 ml tube containing EDTA.

### Clinical chemistry

Bialabo diagnostic kits (France) and a fully automated analyzer (Thermo Fisher Scientific, INDIKO, Finland) were used to test serum Aspartate Aminotransferase (AST) and serum Aspartate Alanine-Transferase (ALT). At 0, 7, 14, 28, 42, 60, and 90 days, AST and ALT levels were measured in all animals.

### Histopathological evaluation

The liver was examined for gross pathological changes and its weight was measured during necropsy. The liver was serially cut, and the cut surfaces were photographed with a reference number and examined for any changes in color, necrosis, or fibrosis. Two random slices were chosen at random and immediately fixed in ten times the volume of the slices in 10% formalin for routine histological evaluation. If any abnormal areas were found, additional slices were fixed in the same way for histological examination. The observed changes were recorded in the datasheet. Following processing and

dehydration, prepared slides were transferred to xylene for 5 min, absolute alcohol for 5 min, 90% alcohol for 2 min, 80% alcohol for 2 min, 70% alcohol for 2 min, 60% alcohol for 2 min, and stained with hematoxylin and eosin. Periodic Acid – Schiff stain (PAS) and silver special stains were used as special stains. Due to the short duration of the experiment, the Masson trichrome was not used to assess fibrosis. The prepared sections were examined for histological changes using a light microscope (Olympus CX31, magnifications x40 x100 and x400). The scoring system consisted of evaluating the tissues and assigning a semi-quantitative score scheme ranging from 0 to 3 based on changes observed in the liver architecture, hepatocytes, and the presence of inflammation. Following an initial review, selected tissues were re-evaluated.

### Statistical evaluation

The statistical significance was determined using one-way ANOVA and the t-test in MINITAB version 17 statistical software (MINITAB, State College, PA, USA).

## Results

### Clinical findings

The physical appearance of the MC-LR treated animals did not differ from that of the controls, and no signs of toxicity were observed during the study.

The mean body weight of both the MC-LR treated and control groups increased until the twelfth week, then decreased (Figure 2). At 13 and 14 weeks of exposure, rats received HW and W concentrations of MC-LR that were significantly lower (p=0.000) than controls, resulting in a decreasing trend in body weight across dose (Figure 2).

There were no obvious pathological changes in the livers. The relative livers of MC-LR treated rats were less than those of control rats, and the difference was statistically significant at all dose levels (p=0.000). The absolute weight reduction differed significantly in the MC-LR treated groups at 0.091 g/kg and 0.105 g/kg (p=0.000) (Table 1).

### Hematology

Hematological parameters for toxicity study of MC-LR measured are summarized in Table 2. The MC-LR treated HW (0.105 g/kg) groups showed a treatment-related erythron effect, as evidenced by decreases in hematocrit and hemoglobin concentrations. The MC-LR treated EN (0.091 g/kg) groups showed a treatment-related erythron effect, as evidenced by decreases in hematocrit and hemoglobin concentrations. There was a significant dose-related decrease in the number of monocytes in the MC-LR treated EN (0.091 g/kg) group (p=0.008), while lymphocyte counts decreased significantly in the MC-LR treated EN (0.091 g/kg) group (p=0.003).

### Clinical chemistry

At day 28, 42, 60, and 90 of the MC-LR treatment, AST activity increased significantly in the HW (0.105 g/kg) (p=0.000), W (0.070 g/kg) (p=0.000), LW (0.035 g/kg) (p=0.000), and EN (0.091 g/kg) (p=0.000) groups compared to the control group (Figure 3).

At days 42, 60, and 90, the HW (0.105 g/kg) (p=0.000), W (0.070 g/kg) (p=0.000), LW (0.035 g/kg) (p=0.000), and EN (0.091 g/kg) (p=0.000) groups had significantly higher ALT activity than the control group (Figure 4).

### Histopathology

Histopathological examination of the livers revealed microscopic lesions associated with MC-LR exposure, as shown in Table 3.

The hepatocytes showed mild sinusoidal congestion (Figure 5.1b), mild vascular congestion (Figure 5.1c), mild lobular inflammation (Figure 5.1d), mild hemorrhage (Figure 5.1e) and severe microvesicular steatosis (Figure 5.1f) in HW (0.105 µg/kg). Mild lobular inflammation (Figure 5.2c), mild hemorrhage (Figure 5.2d), mild perivenular inflammation (Figure 5.2e), severe Kupffer cells (Figure 5.2b), severe microvesicular steatosis (Figure 5.2f) were included in EN (0.091 µg/kg) group. Mild lobular inflammation (Figure 5.3b) and mild microvesicular steatosis (Figure 5.3c) were in W (0.070 µg/kg).

## Discussion

Significant changes in organ/body weight ratios, clinical chemistry, and histology were seen in 90 days of oral administration of MC-LR. After the 12th week, MC-LR treated groups were showed a dose-related reduction in weight increase [30]. Manage et al. was observed a progressive increase in mean body weights in Wister rats after oral administration of toxic *M. aeruginosa* (PCC 7820) up to the 10th week of treatment, followed by a fall in body weight [31]. Milutinović et al. was found a substantial reduction in mean body weight in the MC-LR treated rat group ($p<0.05$). Hepatotoxicity [32], gastrointestinal toxicity [33], neurotoxicity [33], and reproductive toxicity [32] have all been confirmed in previous studies. The results show that oral treatment of MC-LR caused functional hepatotoxicity in rats, with statistically significant differences in absolute and relative weight decrease in the 0.091 g/kg and 0.105 g/kg MC-LR treated groups (p=0.000).

Several indicators in the serum study also indicated liver impairment. Increased serum levels of AST and ALT activity was

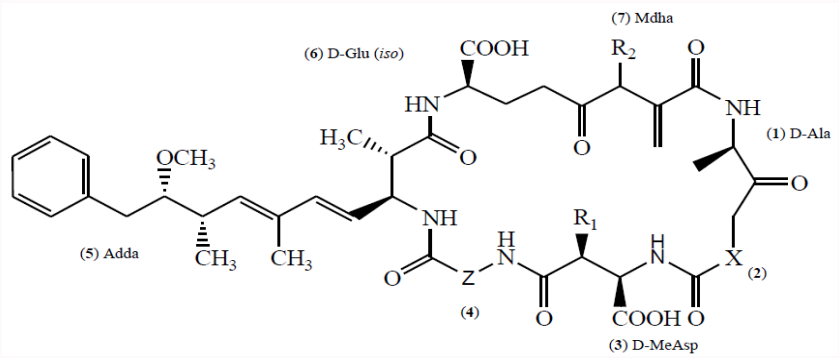


**Figure 1:** General structure of Microcystin, adapted from [4]. In MC-LR X represents L-Leucine; Z L-Arginine; R1 and R2 CH3.

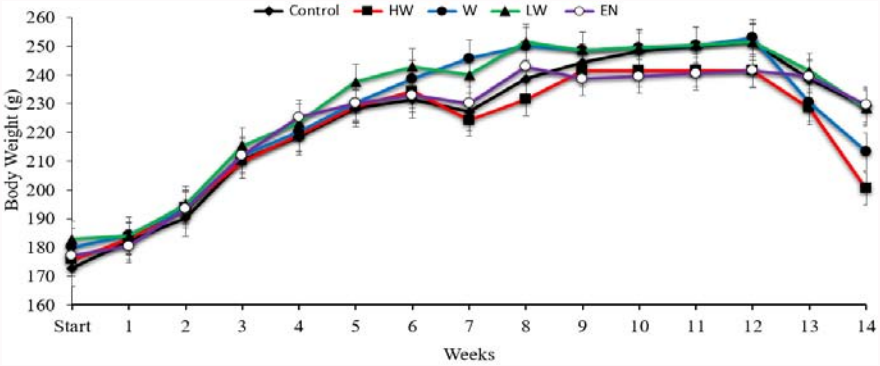


**Figure 2:** Mean body weight changes of Wistar rats in different MC-LR doses (b) (HW= 0.105 µg/kg, W= 0.070 µg/kg, LW= 0.035 µg/kg, EN= MC-LR at Environmental Sample (0.091 µg/kg).

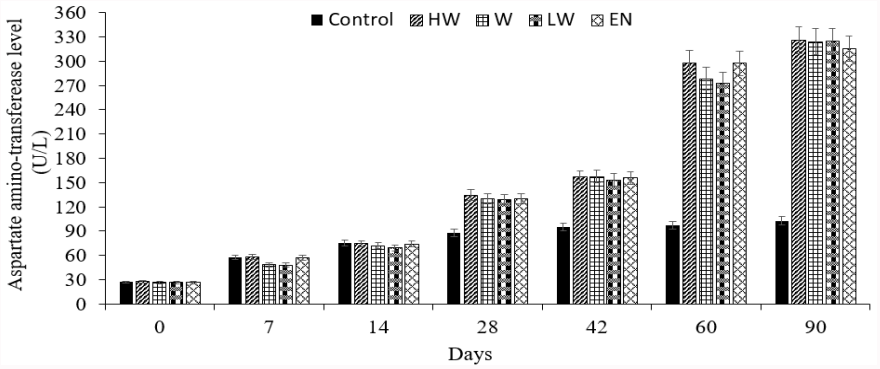


**Figure 3**: Mean serum Aspartate Aminotransferase (AST) levels of Wistar rat groups treated with different MC-LR (b) (HW=0.105 µg/kg, W=0.070 µg/kg, LW=0.035 µg/kg, EN=MC-LR at Environmental Sample (0.091 µg/kg) concentrations.

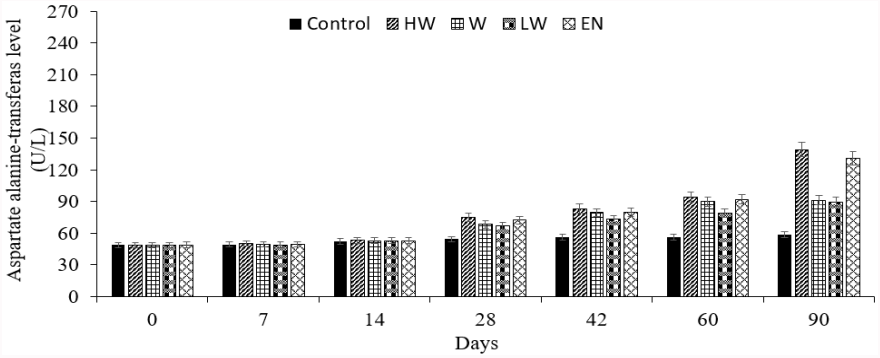


**Figure 4**: Mean Aspartate Alanine-transferase (ALT) levels of Wistar rat groups treated with different MC-LR (b) (HW= 0.105 µg/kg, W= 0.070 µg/kg, LW= 0.035 µg/kg, EN= MC-LR at Environmental Sample (0.091 µg/kg) concentrations.

indicated liver toxicity, which was consistent with histological abnormalities. In the MC-LR exposure 0.105 g/kg, 0.070 g/kg, 0.035 g/kg, and 0.091 g/kg groups, mean serum AST and ALT activity was increased, indicating hepatocellular damage. Analyses of blood markers (ALT, AST) that roughly represent liver function are included in the biochemical examinations. Towards the end of the experiment, the serum ALT activity increased. Aspartate aminotransferase (AST) is a less specific indication of liver injury than ALT since it is found in both the cytoplasm and the mitochondria [30]. Other research was found that children exposed to MCs through drinking water and aquatic food in the Three Gorges Reservoir Region have a higher mean AST value [18]. In another study, chronically exposed fishermen's serum MC concentrations had a larger positive connection with ALT and AST levels than with other biochemical indices, implying that MC buildup could impact the activity of these serum enzymes, which are indications of liver function [34].

In the MC-LR treated 0.091 µg/kg group, a significant reduction in the erythrocyte count (P=0.002), lymphocyte count (P=0.000), and monocyte count (P=0.001). There were no significant differences in the hemoglobin values and platelet counts among experimental groups. On the other hand, RBC count, hematocrit value, MCH, MCV, and MCHC showed significant differences in groups compared with the control group. The most profound changes were observed that showed the highest drop in RBC count and subsequent

**Table 1:** Bodyweight (g), absolute weight (g), and relative percentage of liver weight of male Wistar rats exposed to different doses of MC-LR in gavage for 90 days.

| Body/Liver weight (g)/% | Control | Pure MC-LR Dose (µg/kg) | | | MC-LR Environmental dose (µg/kg) | *p* value |
|---|---|---|---|---|---|---|
| | 0 | 0.035 (LW) | 0.070 (W) | 0.105 (HW) | 0.091 (EN) | |
| **Body weight (g)** | 229 | 200* | 213* | 228* | 229* | *p<0.05* |
| | 14.36, 7 | 11.27, 7 | 4.69, 7 | 5.79, 7 | 11.49, 7 | |
| **Absolute weight (g) of Liver** | 6.16 | 5.11 | 5.05 | 5.15* | 4.49* | *p<0.05* |
| | 1.05, 7 | 0.90, 7 | 1.32, 7 | 0.45, 7 | 0.49, 7 | |
| **% Liver** | 3.04 | 2.81* | 2.71* | 2.62* | 2.40* | *p<0.05* |
| | 0.25, 7 | 0.33, 7 | 0.52, 7 | 0.52, 7 | 0.25, 7 | |

Data are mean, standard deviation, number of rats, **p<0.05*

**Table 2:** Selected Hematology data for Wistar Rats in the 90-days sub-chronic toxicity study of MC-LR.

| Group | HW (0.105 µg/kg) | W (0.070 µg/kg) | LW (0.035 µg/kg) | EN (0.091 µg/kg) | Control |
|---|---|---|---|---|---|
| **Hematocrit (%)** | 27.88 ± 8.09 | 63.90 ± 14.89 | 53.60 ± 8.13 | 33.70 ± 3.31 | 33.70 ± 3.31 |
| **Hemoglobin (g/dL)** | 11.30 ± 1.35 | 20.74 ± 4.45 | 20.12 ± 5.85 | 9.40 ± 2.46 | 16.23 ± 1.02 |
| **WBC ($10^9$/L)** | 4.88 ± 3.83 | 3.03 ± 0.96 | 2.35 ± 1.11 | 7.04 ± 2.89 | 3.10 ± 1.55 |
| **RBC ($10^{12}$/L)** | 8.45 ± 3.25 | 11.77 ± 2.61 | 5.40 ± 1.52 | 9.72 ± 1.96 | 6.47 ± 0.59 |
| **Mean Cell Volume (fL)** | 105.44 ± 4.56 | 108.37 ± 2.64 | 51.54 ± 1.25 | 111.07 ± 9.23 | 104.17 ± 2.46 |
| **MCHC (g/dL)** | 65.28 ± 3.39 | 62.73 ± 1.92 | 33.94 ± 1.08 | 60.13 ± 4.64 | 67.00 ± 3.36 |
| **PLT ($10^9$/L)** | 500.00 ± 122.44 | 60.00 ± 56.14 | 43.00 ± 37.94 | 755.20 ± 408.05 | 120.67 ± 130.88 |
| **Lymphocyte count ($10^9$/L)** | 1.97 ± 1.02 | 3.84 ± 2.80 | 5.48 ± 2.69 | 1.85 ± 0.82* | 2.20 ± 1.13 |
| **Monocyte count ($10^9$/L)** | 0.20 ± 0.00 | 0.56 ± 0.54 | 0.72 ± 0.30 | 0.20 ± 0.00* | 0.30 ± 0.14 |
| **Granulocyte count ($10^9$/L)** | 0.48 ± 0.52 | 0.40 ± 0.11 | 0.35 ± 0.30 | 0.84 ± 0.46 | 0.60 ± 0.28 |

alterations in all index's dependent on the count of erythrocytes such as MCV, MCH, and MCHC. The life cycle of erythrocytes varies from 80 to 120 days. Only a limited number of studies reported the impacts of cyanotoxins on hematological and biochemical parameters in rats or mice. There are mostly reports regarding the rise of liver enzymes [35,36] defined an enzyme increase. Interestingly, similar variations in the red blood cells were originate in fish after exposure to cyanotoxins [37]. Considering the immunological parameters, there were changes in lymphocyte subpopulations in groups of high doses of microcystin and feed with meat from carps caught in cyanobacterial bloom-polluted pond.

The signs of hepatic injuries due to MC-LR were further confirmed by the present study. Liver/body weight ratios, the elevation of AST and ALT levels, and histopathological changes were important parameters of MC-LR-induced liver toxicity.

The hepatocytes showed mild sinusoidal congestion, mild vascular congestion, mild lobular inflammation, mild hemorrhage, and severe microvesicular steatosis in MC-LR exposure 0.105 µg/kg group. Mild lobular inflammation, mild hemorrhage, mild perivenular inflammation, severe Kupffer cells, severe microvesicular steatosis were included in MC-LR exposure 0.091 µg/kg group. Mild lobular inflammation and mild microvesicular steatosis were in MC-LR exposure 0.070 µg/kg group.

Liver tissue preparations from the MCLR-treated group presented cytoplasmic vacuolization and apparent broad hepatocellular gaps. The transmission electron microscopy images displayed that the sections derived from the control group had distinct cell junctions, intact plasmalemma and intact nuclei with complete nuclear membranes. However, there were prominent morphologic changes in the MCLR-treated group. The current study validated the symptoms of hepatic damage caused by MC-LR. Important measures of MC-LR-induced liver damage were liver/body weight ratios, AST and ALT elevations, and histological alterations.

## Conclusion

The findings of the present study revealed that the degree of liver damage was linked to the dose of MC-LR delivered. It provides considerable supportive evidence for the assertion that consumption of water contaminated with MC-LR can cause liver cell damage in mammals.

## Ethics Approval and Consent to Participate

Ethical approval for all experiment procedures were obtained from the Ethics Review Committee of the Faculty of Medical Sciences, (An.E.17/18), University of Sri Jayewardenepura.

## Acknowledgement

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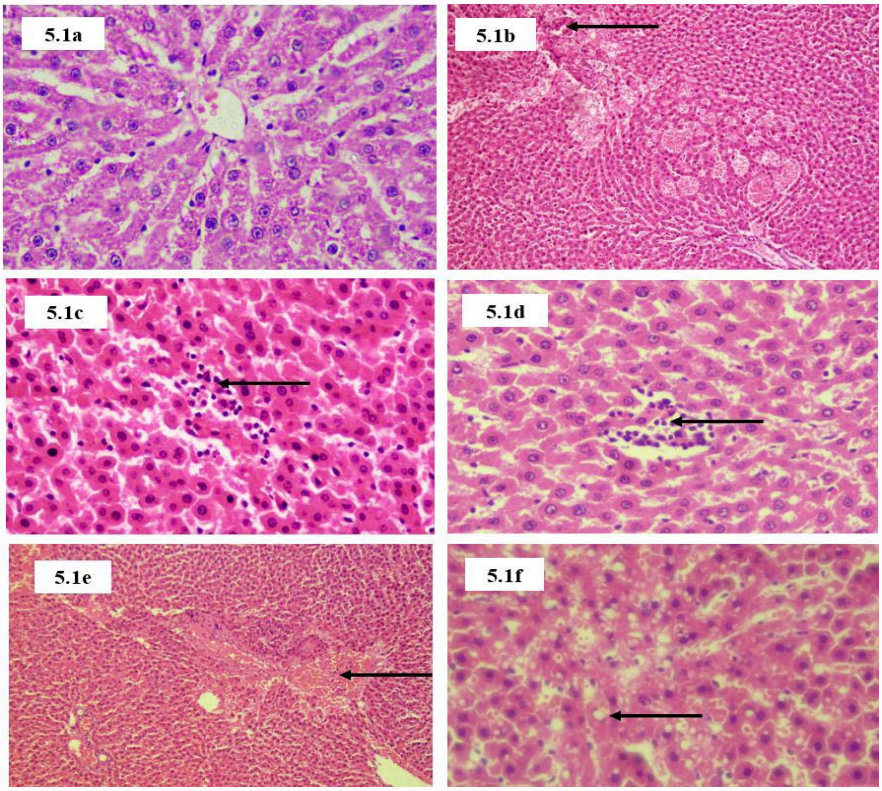


**Figure 5.1:** Above WHO level (HW) Hematoxylin and eosin stain
5.1a) Control x400, 5.1b) sinusoidal congestion x100, 5.1c) Vascular congestion (arrow) x400, 5.1d) Lobular inflammation x400, 5.1e) Hemorrhage x100, 5.1f) Microvesicular steatosis x400

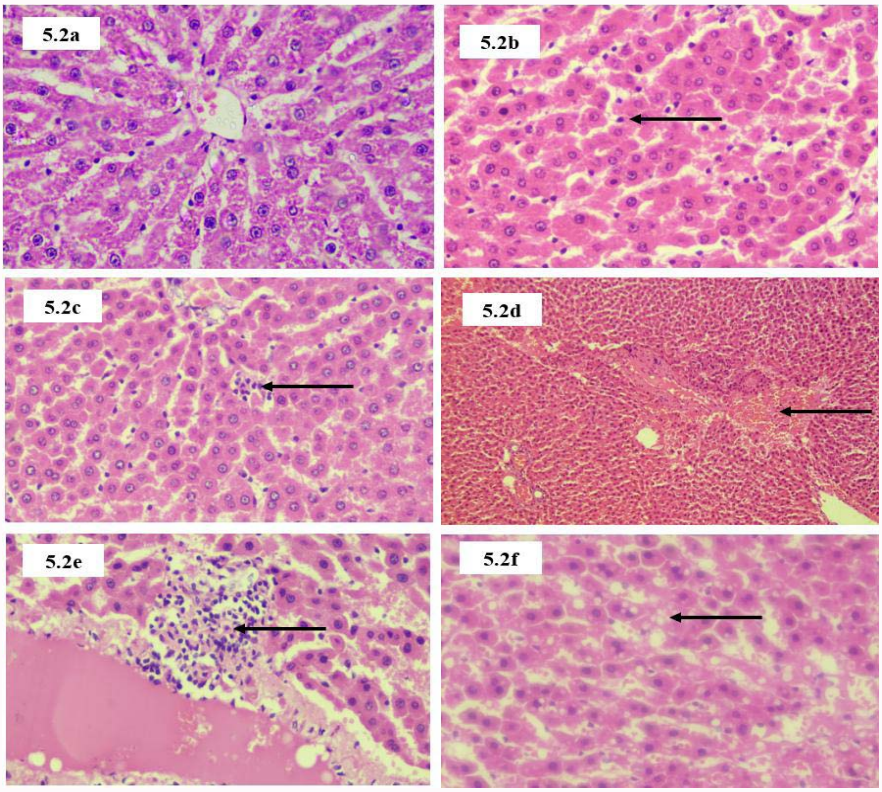


**Figure 5.2:** Environmental exposure (EN) Hematoxylin and eosin stain.
5.2a) Control x400, 5.2b) Prominent Kupffer cells x400 5.2c) Lobular inflammation x400, 5.2d) Hemorrhage x100, 5.2e) Perivenular inflammation x400, 5.2f) Microvesicular steatosis x400

**Table 3:** Incidence and severity of histopathologic liver lesions in Wistar rats in the sub-chronic toxicity study of MC-LR.

| Group | HW | W | LW | EN | Control |
|---|---|---|---|---|---|
| | **(0.105 µg/kg)** | **(0.070 µg/kg)** | **(0.035 µg/kg)** | **(0.091 µg/kg)** | |
| **Histological features** | | | | | |
| **Hepatocytes** | Normal | Normal | Normal | Normal | Normal |
| | **(0)** | **(0)** | **(0)** | **(0)** | **(0)** |
| **Inflammation-lobular** | Mild/spotty inflammation | Mild/spotty inflammation | Normal | Mild/spotty inflammation | Normal |
| | <5/lpf **(1)** | <5/lpf **(1)** | **(0)** | <5/lpf **(1)** | **(0)** |
| **Inflammation- perivenular** | Normal | Normal | Normal | Mild/spotty inflammation | Normal |
| | **(0)** | **(0)** | **(0)** | <5/lpf **(1)** | **(0)** |
| **Hepatocyte cell death** | Normal | Normal | Normal | Normal | Normal |
| | **(0)** | **(0)** | **(0)** | **(0)** | (0) |
| **Accumulation of pigment** | Normal | Normal | Normal | Normal | Normal |
| | **(0)** | **(0)** | **(0)** | **(0)** | **(0)** |
| **Hepatocyte regeneration** | Normal | Normal | Normal | Normal | Normal |
| | **(0)** | **(0)** | **(0)** | **(0)** | **(0)** |
| **Kupffer cells** | Normal | Normal | Normal | Severe | Normal |
| | **(0)** | **(0)** | **(0)** | **(3)** | **(0)** |
| **Nuclear pyknosis** | Normal | Normal | Normal | Normal | Normal |
| | **(0)** | **(0)** | **(0)** | **(0)** | **(0)** |
| **Hemorrhage** | Mild | Normal | Normal | Mild | Normal |
| | **(1)** | **(0)** | **(0)** | **(1)** | **(0)** |
| **Sinusoidal congestion** | Mild | Normal | Normal | Normal | Normal |
| | **(1)** | **(0)** | **(0)** | **(0)** | **(0)** |
| **Vascular congestion** | Mild | Normal | Normal | Normal | Normal |
| | **(1)** | **(0)** | **(0)** | **(0)** | **(0)** |
| **Microvesicular steatosis** | Severe | Mild | Normal | Severe | Normal |
| | **(3)** | **(1)** | **(0)** | **(3)** | **(0)** |

Lesion severity was based on a 0–3 grading scale, (0-Normal, 1-Mild, 2-Moderate, 3-Severe)

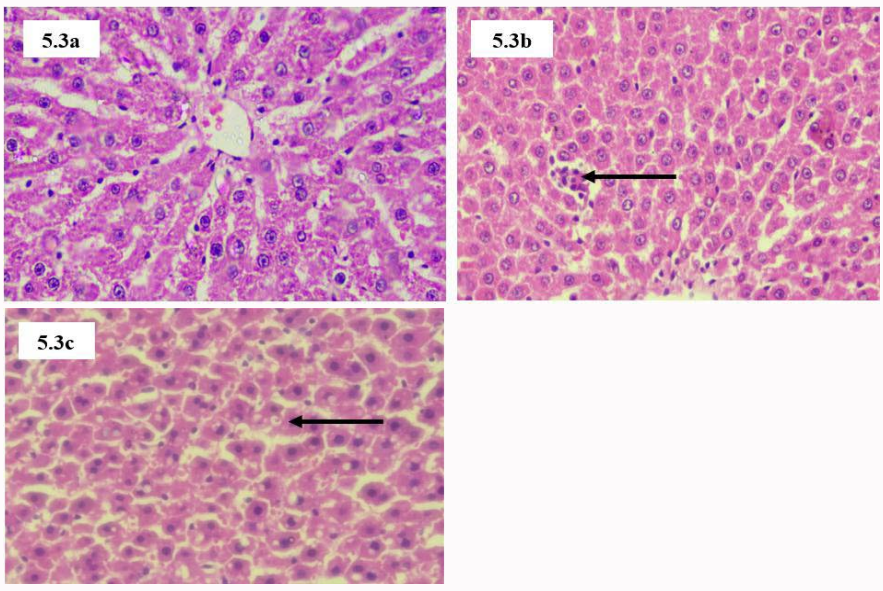


**Figure 5.3:** WHO level (W). Hematoxylin and eosin stain
5.3a) Control - x400, 5.3b) Lobular inflammation x400, 5.3c) Microvesicular steatosis x400

## References


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