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# **Nephrotoxicity of Cylindrospermopsin (CYN) and Microcystin-LR (MC-LR) on Mammalian Kidney: Wistar Rat as a Model Assessment**

**H.A.S.N. Abeysiri\*(\*\*), J.K.P. Wanigasuriya\*\*\*, T.S. Suresh\*\*\*\*, D.H. Beneragama\*\*\*\*\* and P.M. Manage\*†** \*Centre for Water Quality and Algae Research, Department of Zoology, University of Sri Jayewardenepura, Sri Lanka \*\*Faculty of Graduate Studies, University of Sri Jayewardenepura, Sri Lanka

\*\*\*Centre for Kidney Research, Department of Medicine, Faculty of Medical Sciences,

University of Sri Jayewardenepura, Sri Lanka

\*\*\*\*Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka \*\*\*\*\*Department of Pathology, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka

†Corresponding author: Pathmalal M. Manage; pathmalal@sjp.ac.lk

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# **ABSTRACT**

Naturally derived cyanotoxins, cylindrospermopsin (CYN), and microcystin-LR (MC-LR) have shown hepatotoxic and nephrotoxic effects in several studies. The present study aimed to determine the possible nephrotoxicity of MC-LR and CYN on mammalian kidneys using male Wistar rats as an animal model. Potential nephrotoxicity was evaluated at different doses of CYN (0.175 µg.kg<sup>-1</sup>, 0.140 µg.kg<sup>-1</sup>, 0.105 µg.kg<sup>-1</sup>) and MC-LR (0.105 µg.kg<sup>-1</sup>, 0.070  $\mu$ g.kg<sup>-1</sup>, 0.035  $\mu$ g.kg<sup>-1</sup>) was observed. Water samples from dug wells contaminated with CYN  $(0.161 \mu g kg^{-1})$  and MC-LR  $(0.091 \mu g kg^{-1})$  from the Padaviya area in Anuradhapura, Sri Lanka were used as environmental samples. The control groups were treated with distilled water. The exposure time of rats to the toxin was 90 days. Evaluation of urinary creatinine, serum creatinine, and Kidney Injury Molecule-1 (KIM-1) were estimated using standard protocols. A significant increase in serum creatinine levels was observed in all CYN and MC-LR treated groups (*p*<0.05) after 7 and 42 days of exposure, respectively, compared to control. It was found a decrease of urine creatinine when rats were treated with different concentrations of CYN and MC-LR (*p*<0.05) after 7 days compared to the control. The highest KIM-1 concentrations were recorded at 0.175 µg.kg<sup>-1</sup> of CYN and 0.105 µg.kg<sup>-1</sup> of MC-LR. The concentrations of KIM-1 in the control groups for CYN-treated and MC-LRtreated were not detected. Luminal protein, nuclear pyknosis, mild tubular epithelial swelling, vascular congestion, and interstitial inflammation in CYN and MC-LR treated groups were common. No predominant changes were observed in the control groups treated with CYN and MC-LR. The results of the present study confirm that the consumption of CYN and MC-LR-contaminated water may lead to kidney injury in Wistar rats.

# **INTRODUCTION**

Cyanotoxins can accumulate in aquatic wildlife and enter the food chain (Lance et al. 2007), resulting in the risk of human and livestock poisoning. Among different cyanotoxins, cylindrospermopsin (CYN) and Microcystins (MCs) are the most dominant and toxic in the aquatic environment (Sethunga & Manage 2010, Wijewickrama & Manage 2019). MCs and CYN have heat-stable chemical structures and cannot be removed even when heated at 100°C (Lawton et al. 2011, Manage 2019). MC-LR is the most dominant and toxic MC variant among over 100 MC congeners: MC-LR, -RR, -LW, -YR, -LA, etc. (Du et al. 2019). The endotoxin CYN is another commonly found hydrophilic alkaloid with a stable chemical structure. Colonial cyanobacteria produce MC-LR; *Microcystis* spp., *Planktothrix*, and the filamentous *Anabaena, Gloeotrichia, Nodularia, Oscillatoria,* and *Nostoc* sp. whereas CYN is produced by non-bloom-forming cyanobacteria *Cylindrospemospisraciborskii*, *Raphidiopsis curvata, Lyngbya whole, Umezakia natans, Anabaena bergii, Aphanizomenon flos-aquae, Aphanizomenon ovalisporum, Anabaena lapponica* (Foss & Aubel 2013). Considering the toxic nature and the influence of toxicity on human health, the World Health Organization (WHO) established a provisional limit of 1  $\mu$ g.L<sup>-1</sup> for MC-LR and 2  $\mu$ g.L<sup>-1</sup> for CYN in drinking water (WHO 2003). Further Tolerable Daily Intake (TDI) for humans was established as  $0.04 \mu$ g.kg<sup>-1</sup> of body weight/day for MC-LR (Do Carmo Bittencourt-Oliveira et al. 2016) and 0.02  $\mu$ g.kg<sup>-1</sup> of body weight/day for CYN (Guzmán-Guillén et al. 2014).

MCs are generally considered hepatotoxins. Several studies have shown MC-LR convinced hepatotoxicity by inhibiting protein phosphatase 1 and 2A (PP1 and PP2A) and inducing the production of reactive oxygen species (ROS), followed by destroying the cell cytoskeleton, which eventually leads to liver cell necrosis and apoptosis (Clark et al. 2007). In recent times, MCs have been shown to penetrate renal cells in organic anion-transporting polypeptides (OATPs) dependent manner, subsequently promoting the accumulation of MC-LR (Jia et al. 2014). Therefore, exposure to MCs may cause toxicity in the kidney (Menezes et al. 2013). Exposure to MC-LR could induce apoptosis in both human embryonic kidney (HEK-293) and human kidney adenocarcinoma (ACHN) cell lines by decreasing the G2/M phase population (Piyathilaka et al. 2015).

CYN has become a public health concern following reports of "mysterious disease" events on Palm Island, Australia (Chernoff et al. 2018). CYN is a 415 Da alkaloid, stable over large ranges of pH and temperatures. Singledose intraperitoneal administration of 0.2 mg CYN.kg<sup>-1</sup> bw revealed that the renal excretory pathway plays a major role and is responsible for 65.2% of the dose excretion (Moraes & Magalhães 2018). Chernoff et al. (2018) revealed that intraperitoneal administered CYN in mice affects intense membrane proliferation in convoluted proximal tubule cells. Necrotic cells and cytoplasmic fat droplets have been reported in both the proximal and distal tubules. Renal toxicity was well-defined following the treatment of male Swiss mice with toxic cellular extracts following both single oral dose and intraperitoneal administrations; the effects contained large cytoplasmic vacuoles and proximal apical vesicles and the distal tubule clog with proteinaceous material (Moraes & Magalhães 2018). Other studies used single oral dose exposure of toxic cellular extracts to confirm intense cytoplasmic vacuolization and increased lumen and protein contents in the proximal tubular cells (Chernoff et al. 2018).

MCs (MC-LR) and CYN are being hypothesized as one of the risk factors for Chronic Kidney Disease of unknown etiology (CKDu) in Sri Lanka (Abeysiri et al. 2018a, Abeysiri et al. 2018b, Manage, 2019, Piyathilaka et al. 2015). The highest prevalence of CKDu is reported in the North Central Province of Sri Lanka and emerging in the Uva, Eastern, and North Western Provinces (Chandrajith et al. 2011). The disease is unrelated to conventional risk factors of chronic kidney disease (CKD), and the pathology is consistent with tubule-interstitial nephritis (Wijethunga et al. 2015). The impact of cyanotoxins on kidneys has become a research interest as cyanobacteria were found in 75% of freshwater bodies tested in CKDu highly prevalent areas (Manage 2019). Studies have further demonstrated that surface and

dug well water in CKDu endemic areas used for drinking and irrigation has a significant relationship between the cell density of cyanobacteria and cyanotoxins (Abeysiri et al. 2018a). These findings favor the hypothesis that cyanotoxininduced nephrotoxicity is one of the possible explanations for CKDu prevalence (Abeysiri et al. 2018a, 2019b, 2019a, Manage 2019). The objective of this study was to determine the possible effects of CYN and MC-LR on mammalian kidneys using male Wistar rats as the animal model.

# **MATERIALS AND METHODS**

# **Animals**

Eight-week-old male Wistar rats were purchased from the Medical Research Institute (MRI), Sri Lanka. Animals were acclimatized for one week and maintained in the Animal House, Faculty of Medical Sciences, the University of Sri Jayewardenepura, on a 12h: 12h light-dark cycle at  $28 \pm 2^{\circ}$ C. Food and water were available ad libitum. Ethics approval was obtained from the Ethics Review Committee of the Faculty of Medical Sciences (No.17/18, ERC, FMS, USJ), University of Sri Jayewardenepura.

# **Experimental Design for CYN and MC-LR Chronic Exposure Study**

In the present study, two experiments were conducted to evaluate the nephrotoxicity of the cyanotoxins CYN and MC-LR. For each experiment, thirty-five rats were divided into five experimental groups. One set of groups was for CYN, with the following dosages: HW-0.175  $\mu$ g.kg<sup>-1</sup>, W-0.140  $\mu$ g.kg<sup>-1</sup>, LW-0.105  $\mu$ g.kg<sup>-1</sup>, EN-0.161  $\mu$ g.kg<sup>-1</sup>, and a control group. Another set of thirty-five rats was divided into five experimental groups for MC-LR, with dosages of HW-0.105  $\mu$ g.kg<sup>-1</sup>, W-0.070  $\mu$ g.kg<sup>-1</sup>, LW-0.035  $\mu$ g.kg<sup>-1</sup>, EN- $0.091 \mu$ g.kg<sup>-1</sup>, and a control group and each group comprised seven rats. In the CYN exposure study, rats were treated at 0.175  $\mu$ g.kg<sup>-1</sup>, 0.140  $\mu$ g.kg<sup>-1</sup>, and 0.105  $\mu$ g.kg<sup>-1</sup>, respectively. CYN-contaminated water was orally administered to individual rats for oral feeding using a Sondi needle during the study period of 90 days. The environmental exposure (EN) group received environmental water samples contaminated with CYN  $(0.161 \mu g.kg^{-1})$  obtained from a randomly selected well in Padaviya.

For the MC-LR exposure study, rats received the toxin as 0.105  $\mu$ g.kg<sup>-1</sup>, 0.070  $\mu$ g.kg<sup>-1</sup>, and 0.035  $\mu$ g.kg<sup>-1</sup>. MC-LR contaminated water was orally administered to individual rats for oral feeding using a Sondi needle during the study period of 90 days. The Environmental exposure (EN) group received an environmental water sample contaminated with MC-LR  $(0.091 \mu g/kg^{-1})$  obtained from a randomly selected well in Padaviya.

Finally, the absolute and relative weights of the left and right kidneys were calculated. Relative weight was calculated using the following equation.

Relative weight (%) = (Organ weight/Body weight)  $\times$  100

#### **Blood Collection**

The venous blood samples were collected from the lateral tail veins of each rat at 0, 7, 14, 28, 42, and 60 days. Blood was transferred to 1.5 mL Eppendorf tubes and centrifuged at 13,000 rpm for 10 min. to separate the serum. The serum was stored at -80°C. At the end of the experiment (90 days), rats were anesthetized, and blood was collected by direct heart puncture for both chemical analyses (Karp et al. 2023).

#### **Urine Collection and Analysis**

Urine was collected from each rat at 0, 7, 14, 28, 42, 60, and 90 days using the metabolic cages and stored at -80°C (Wajda et al. 2020). Kidney Injury Molecule-1 (KIM-1) in urine was analyzed weekly using Elabscience diagnostic kits. This kit recognized Rat KIM-1 in samples. No significant crossreactivity or interference between Rat KIM-1 and analogs was observed (Sensitivity:  $18.75$  pg.mL<sup>-1</sup>, Detection range: 31.25-2000 pg.mL<sup>-1</sup>, Repeatability: Coefficient of variation is  $< 10\%$ ).

Serum creatinine and urine creatinine were analyzed using Bialabo diagnostic kits (France) and a fully automated analyzer (Thermo Fisher Scientific, INDIKO, Finland).

#### **Histopathological Evaluation**

Each rat was sacrificed after 90 days, and both kidneys of each rat were bi-valved and then placed in 10% formalin for fixation. The fixed kidneys were paraffin-embedded, sectioned at 5  $\mu$ m, and stained with hematoxylin and eosin. The prepared sections were examined under the light microscope (Olympus CX31, magnification  $\times$ 40  $\times$ 100 and  $\times$ 400) for any histological changes. After an initial review, selected tissues were re-evaluated.

#### **Statistical Evaluation**

The data was expressed as the mean  $\pm$  standard deviation (SD). All statistical analyses were carried out via MINITAB version 17 statistical software (MINITAB, State College, PA, USA). One-way ANOVA was used to analyze the difference between groups, and differences were considered significant if *p* < 0.05.

#### **RESULTS AND DISCUSSION**

#### **Clinical Findings**

The physical appearance of CYN-treated and MC-LR-treated

animals did not differ from controls, and no signs of toxic effects were observed throughout the study.

Increment of mean body weight in CYN treated and a control group of animals was recorded until the fourteenth week; afterward, body weight was comparable to the control group with an increment of weight (Fig. 1a). A decrease in body weights of rats treated with  $0.175 \mu g.kg^{-1}$ ,  $0.140 \mu g.kg^{-1}$ , and 0.161  $\mu$ g.kg<sup>-1</sup> concentrations of CYN, compared to the control group, was found at 12, 13, and 14 weeks  $(p > 0.05)$ . The mean body weight of MC-LR treated and control groups increased until the twelfth week and after that decreased (Fig. 1b). A decreasing trend in body weight across doses was recorded when rats received  $0.105 \mu g \text{.kg}^{-1}$  and 0.091  $\mu$ g.kg<sup>-1</sup> concentrations of MC-LR which were lower than controls at 13 and 14 weeks of exposure (Fig. 1b). Increment of body weight with low CYN doses (30 and 60  $\mu$ g.kg<sup>-1</sup>.day<sup>-1</sup>) and decrease of body weight at high CYN (432 and 657  $\mu$ g.kg<sup>-1</sup>.day<sup>-1</sup>) in mice study was recorded by Humpage and Falconer (2003). The gradual increase of mean body weights of the Wister rat model with oral administration of toxic *M. aeruginosa* (PCC 7820) was recorded up to the 10<sup>th</sup> week treatment, and after that decline of body weight was recorded by Manage et al. (2009) as well. A significant reduction of mean body weight following the MC-LR treated rat group (*p*<0.05) was recorded by Milutinović et al. (2003). The hardening of the glomeruli is often a feature of CKDu, which leads to a reduction in kidney size as the disease progresses. CYN is known to affect both hepatic and renal systems (Bazin et al. 2012), and some studies have confirmed hepatotoxicity (Lone et al. 2015), gastrointestinal toxicity (Wu et al. 2018), neurotoxicity (Wu et al. 2018), and reproductive toxicity (Lone et al. 2015) of MC-LR. However, the studies on nephrotoxicity induced by prolonged oral exposure to MC-LR and CYN are limited.

The absolute and relative weights of the right and left kidneys of CYN and MC-LR-treated rats were less than the control, and the difference was statistically significant  $(p < 0.05)$  in 0.175  $\mu$ g.kg<sup>-1</sup> and 0.161  $\mu$ g.kg<sup>-1</sup> of CYN-treated rats. The reduction of relative weight differed significantly in 0.105  $\mu$ g.kg<sup>-1</sup> and 0.091  $\mu$ g.kg<sup>-1</sup> of MC-LR treated rats, and the reduction of absolute weight significantly differed from only the 0.091  $\mu$ g.kg<sup>-1</sup> MC-LR treated group ( $p < 0.05$ ) (Table 1).

Chernoff et al. (2018) showed significant dose-related increases ( $p < 0.05$ ) in absolute and relative weights of kidneys in male mice, and Manage et al. (2009) reported the mean absolute weight of kidneys among MC-LR treated animals was not statistically significant (*p* < 0.33) and relative weights of kidneys did not differ significantly. The weight loss of kidneys is a common cause of acute kidney



EN; 0.161 µg.kg<sup>-1</sup> and MC-LR treatments) (b) (HW; 0.105 µg.kg<sup>-1</sup>, W; 0.070 µg.kg<sup>-1</sup>, LW; 0.035 µg.kg<sup>-1</sup>, EN; 0.091 µg.kg<sup>-1</sup>). Fig. 1: Mean body weight changes of Wistar rats in different CYN doses (a) (HW;  $0.175 \mu g.kg^{-1}$ , W;  $0.140 \mu g.kg^{-1}$ , LW;  $0.105 \mu g.kg^{-1}$ ,

Table 1: Absolute weight (g) and relative percentage of right and left kidneys of male Wistar rats exposed to oral administration of different doses of CYN and MC-LR for 90 days.

Body/Kidney weight $\left[\frac{g}{g}\right]$ /%	<b>CYN</b> Exposure					<b>MC-LR</b> Exposure				
	Control	Pure CYN Dose [ $\mu$ g.kg <sup>-1</sup> ]			<b>CYN</b> Environmental dose [ $\mu$ g.kg <sup>-1</sup> ]	Control	Pure MC-LR Dose [ $\mu$ g.kg <sup>-1</sup> ]			MC-LR Environmental dose [ $\mu$ g.kg <sup>-1</sup> ]
	$\theta$	0.105	0.140	0.175	0.161	$\overline{0}$	0.035	0.070	0.105	0.091
Absolute weight $(g)$	$0.84 \pm$	$0.76 \pm$	0.75	$0.73*$	$0.74*$	0.63	0.51	0.54	0.52	$0.46*$
of Right Kidney	0.08	0.03	$\pm 0.06$	$\pm 0.04$	$\pm 0.04$	$\pm 0.08$	$\pm 0.04$	$\pm 0.06$	$\pm 0.03$	$\pm 0.03$
Absolute weight $(g)$	$0.81 \pm$	$0.79 \pm$	0.77	$0.72*$	$0.72*$	0.59	0.51	0.54	0.53	$0.47*$
of Left Kidney	0.09	0.06	$\pm 0.06$	$\pm 0.04$	$\pm 0.05$	$\pm 0.07$	$\pm 0.04$	$\pm 0.04$	$\pm 0.03$	$\pm 0.04$
% Right Kidney	$0.25 \pm$	$0.23 \pm$	0.21	$0.20*$	$0.18*$	0.29	0.23	0.21	$0.20*$	$0.18*$
	0.01	0.03	$\pm 0.06$	$\pm 0.04$	$\pm 0.04$	$\pm 0.01$	$\pm 0.03$	$\pm 0.06$	$\pm 0.04$	$\pm 0.04$
% Left Kidney	$0.24 \pm$	$0.22 \pm$	0.21	$0.19*$	$0.18*$	0.24	0.22	0.21	$0.19*$	$0.18*$
	0.02	0.06	$\pm 0.06$	$\pm 0.04$	$\pm 0.05$	$\pm 0.02$	$\pm 0.06$	$\pm 0.06$	$\pm 0.04$	$\pm 0.05$

 $p < 0.05$ 

injury and is often reversible. It can be caused by severe nephrotoxic drugs or contrast agents used in imaging. The serum creatinine values showed a significant increment after 7 days of exposure in all CYN-treated groups: 0.175 µg.kg<sup>-1</sup>,

0.140  $\mu$ g.kg<sup>-1</sup>, and 0.161  $\mu$ g.kg<sup>-1</sup> compared to the control (*p* < 0.05) (Fig. 2a).

In MC-LR treated groups, a significant increase of serum creatinine was not found until 42 days of exposure,



EN; 0.161 µg.kg<sup>-1</sup>) and MC-LR (b) (HW; 0.105 µg.kg<sup>-1</sup>, W; 0.070 µg.kg<sup>-1</sup>, LW; 0.035 µg.kg<sup>-1</sup>, EN; 0.091 µg.kg<sup>-1</sup>) concentrations. Fig. 2: Mean serum creatinine levels of Wistar rat groups treated with different CYN (a) (HW; 0.175 µg.kg<sup>-1</sup>, W; 0.140 µg.kg<sup>-1</sup>, LW; 0.105 µg.kg<sup>-1</sup>,

and after that significant increment of the serum creatinine was detected for different MC-LR concentrations (*p* < 0.05) compared to the control group (Fig. 2b).

The present data specified a significant decrease of urinecreatinine in rats after 7 days of exposure to all concentrations of CYN compared to the control  $(p < 0.05;$  Fig. 3a). Also, was found a decrease in urine creatinine when rats were treated with different concentrations of MC-LR  $(0.105 \text{ µg.kg}^{-1})$ , respectively to the control was detected. 0.070  $\mu$ g.kg<sup>-1</sup>, 0.035  $\mu$ g.kg<sup>-1,</sup> and 0.091  $\mu$ g.kg<sup>-1</sup> (Fig. 3b).

The highest serum-creatinine in rats treated with CYN concentration 0.175  $\mu$ g.kg<sup>-1</sup> ( $p < 0.05$ ), following 0.140  $\mu$ g.kg<sup>-1</sup> (*p* <0.05) and 0.105  $\mu$ g.kg<sup>-1</sup> was found. Rats function, which correlated to renal dysfunction. exposed to the  $0.161 \mu g \text{ kg}^{-1}$  showed a significant increment of serum-creatinine  $(p<0.05)$ , lower than the rats that received the high dose of CYN in the study. Decrease in urine creatinine levels in the 0.175  $\mu$ g.kg<sup>-1</sup>, 0.140  $\mu$ g.kg<sup>-1</sup>, and 0.105  $\mu$ g.kg<sup>-1</sup> were found, and the rats exposed to 0.161  $\mu$ g.kg<sup>-1</sup> concentration of CYN in the experiment compared to the control. Significant increment of the serum creatinine levels in rats treated with different concentrations of MC-

t significant increment of the serum creatinine LR was found following the descending order from  $0.105$  $\mu$ g.kg<sup>-1</sup> (*p*<0.05), 0.070  $\mu$ g.kg<sup>-1</sup> (*p*<0.05), and 0.035  $\mu$ g.kg<sup>-1</sup> (*p*<0.05), respectively. Similar to the CYN exposure, the second-highest serum creatinine was recorded in rats exposed to 0.161  $\mu$ g.kg<sup>-1</sup> ( $p < 0.05$ ). Decreases in urine creatinine  $\frac{1}{2}$  and the control ( $p < 0.05$ : Fig. 3a). Also, was levels in the MC-LR treated rat groups were followed by 0.105  $\mu$ g.kg<sup>-1</sup>, 0.091  $\mu$ g.kg<sup>-1</sup>, 0.070  $\mu$ g.kg<sup>-1</sup>, and 0.035  $\mu$ g.kg<sup>-1</sup>, respectively to the control was detected.

In general, an increase in serum creatinine and a decrease hest serum-creatinine in rats treated with in urine creatinine indicate renal damage (Hsu et al. 2020). Rises of the serum creatinine are a significant marker of renal function, which correlated to renal dysfunction (Do Amaral et al. 2008). Yi et al. (2019) recorded no significant changes in the serum creatinine levels in mice treated with MC-LR at different concentrations  $(1, 30, 60, 90, \text{ and } 120 \mu g.L^{-1})$ from 3 and 6 months and Chernoff et al. (2018) recorded no significant alterations in the serum creatinine levels in the mice given CYN at 3 months at different dose levels 0, 75, 150 and 300  $\mu$ g.kg<sup>-1</sup>.d<sup>-1</sup>. However, in the present study, the mean serum creatinine level was raised with a reduction



Fig. 3: Mean Urine creatinine levels of Wistar rat groups treated with different CYN (a) (HW; 0.175 µg.kg<sup>-1</sup>, W; 0.140 µg.kg<sup>-1</sup>, LW; 0.105 µg.kg<sup>-1</sup>, LW; 0.165 µg.kg<sup>-1</sup>, LW; 0.165 µg.kg<sup>-1</sup>, LW; 0.165 µg.kg<sup>-1</sup>, LW; 0. EN; 0.161  $\mu$ g.kg<sup>-1</sup>) and MC-LR (b) (HW; 0.105  $\mu$ g.kg<sup>-1</sup>, W; 0.070  $\mu$ g.kg<sup>-1</sup>, LW; 0.035  $\mu$ g.kg<sup>-1</sup>, EN; 0.091  $\mu$ g.kg<sup>-1</sup>) concentrations.

of urine creatinine levels in the different CYN and MC-LR exposure groups of rats, indicating nephrotoxic injury. When the renal function is normal, these small creatinine molecules are filtered from the glomerulus. However, when the filtering ability of the glomerulus decreases, the concentration of serum creatinine increases. This rise in serum creatinine levels could often be used as an indicator of kidney dysfunction (Chernoff et al. 2011). Reduction of urine creatinine was found in the present study also noted in CYN and MC-LR exposure groups of rats, indicating possible renal injury.

Increment of urinary KIM-1 level was found in CYNtreated rats following descending order from  $0.175 \mu g \text{ kg}^{-1}$  $(35.4 \pm 2.3 - 1328.4 \pm 32.1$  Pg.mL<sup>-1</sup>), 0.140  $\mu$ g.kg<sup>-1</sup> (34.5 ± 2.3-985.3  $\pm$  14.1 Pg.mL<sup>-1</sup>) and 0.105 µg.kg<sup>-1</sup> (35.2  $\pm$  1.6- $456.3 \pm 11.6$  Pg.mL<sup>-1</sup>) respectively. Further, it was found 0.161  $\mu$ g.kg<sup>-1</sup> exposure rat group reported higher KIM-1

atinine levels in the different CYN and MC-level  $(36.3\pm 0.9-1134.2\pm 11.4 \text{ Pg.mL}^{-1})$  than the W and LW dose exposures comparable to the control from weeks 2 to 8 (Fig. 4a). A similar descending pattern of the urinary KIM-1 level was found in MC-LR treated rat groups 0.105 tering ability of the glomerulus decreases, the  $\mu g \cdot kg^{-1}$  (275.1±21.2-1562.1±22.4 Pg.mL<sup>-1</sup>), 0.070  $\mu g \cdot kg^{-1}$  $(164.2\pm12.3-987.1\pm15.4 \text{ Pg.mL}^{-1}), 0.035 \mu\text{g.kg}^{-1} (117.2\pm9.6-1)$ nine levels could often be used as an indicator 993.7 $\pm$ 16.3 Pg.mL<sup>-1</sup>) and high KIM-1 level recorded for 0.091  $\mu$ g.kg<sup>-1</sup> (217.1±16.8-1328.2±18.3 Pg.mL<sup>-1</sup>) than the ine was found in the present study also noted  $0.070 \mu\text{g} \cdot \text{kg}^{-1}$  and  $0.035 \mu\text{g} \cdot \text{kg}^{-1}$  dose exposures compare to control from 4 to 8 weeks of exposure (Fig. 4b).

all injury. CYN-treated groups at two weeks and MC-LR-treated groups at four weeks showed an increasing level of KIM-1.  $\frac{1}{2}$  This was found during 2-8 weeks with the treatment of 0.175  $\mu$ g.kg<sup>-1</sup> This was found during 2-8 weeks with the treatment of 0.175  $\mu$ g.kg<sup>-1</sup> and 0.161  $\mu$ g.kg<sup>-1</sup>, and the delayed effect was found during 3-8 weeks when animal exposure to  $0.140 \text{ µg} \text{ kg}^{-1}$ 14.1 Fig. and 0.105  $\mu$ g.kg (33.2 ± 1.0-<br>S Pg.mL<sup>-1</sup>) respectively. Further, it was found and 0.105  $\mu$ g.kg<sup>-1</sup> of CYN in drinking water. For the MC-LR study, descending order of KIM-1 was found following exposure in group reported ingiter  $\lim_{\epsilon \to \infty} \frac{1}{\epsilon}$  associating order of  $\lim_{\epsilon \to \infty} \frac{1}{\epsilon}$  was found following



Fig. 4: Mean KIM-1 levels of Wistar rats treated with different concentrations of CYN (a) (HW; 0.175  $\mu$ g.kg<sup>-1</sup>, W; 0.140  $\mu$ g.kg<sup>-1</sup>, LW; 0.105  $\mu$ g.kg<sup>-1</sup>,  $W_{\rm eff}$   $W_{\rm eff}$  and  $W_{\rm eff}$ EN; 0.161  $\mu$ g.kg<sup>-1</sup> and MC-LR (b) (HW; 0.105  $\mu$ g.kg<sup>-1</sup>, W; 0.070  $\mu$ g.kg<sup>-1</sup>, LW; 0.035  $\mu$ g.kg<sup>-1</sup>, EN; 0.091  $\mu$ g.kg<sup>-1</sup>).

exposure of 0.105  $\mu$ g.kg<sup>-1</sup>, 0.091  $\mu$ g.kg<sup>-1</sup>, 0.070  $\mu$ g.kg<sup>-1</sup>, and  $0.035 \mu g \cdot kg^{-1}$  during 4-8 weeks.

High concentrations of urinary KIM-1, which is an indication of proximal convoluted tubular damage were  $\frac{1}{2}$  migmentation (Fig. 5c) severe cellular swelling and mo reported from individuals with early stages of CKDu (De Silva et al. 2016). Our study showed increased levels of  $\frac{1}{\text{Fig. 5e)}}$ . Severe cellular swelling and inflamment of KIM-1 in the second week of CYN treatment and the fourth week of the MC-LR treatment compared to the control group, indicating early renal tubular damage. Upregulation of KIM-1 is well-known to occur in proximal tubule damage of the nephron. Increased levels of KIM-1 may also signify its involvement in phagocytosis of damage to the proximal tubule epithelial cells by converting epithelial cells into semi-professional phagocytes (Bonventre et al. 2010, Ichimura et al. 2008). KIM-1 up-regulation may be responsible for restoring the functional and morphological integrity of kidneys as well (Waanders et al. 2010).

#### **Histopathology**

Significant histopathological changes in the  $0.175 \mu g \text{.} \text{kg}^{-1}$ group included mild nuclear pyknosis (Fig. 5b), moderate pigmentation (Fig. 5c), severe cellular swelling and moderate luminal protein (Fig. 5d), and severe glomerular collapse (Fig. 5e). Severe cellular swelling and inflammation (Fig. 6b), severe tubular epithelial swelling and moderate bek of the MC-LR treatment compared to the vascular congestion (Fig. 6c) were significant in 0.161  $\mu$ g.kg<sup>-1</sup> group. Further, it was observed that mild cellular In Of KIM-1 is Well-known to occur in proximal swelling of renal tubules (Fig. 7b, Fig. 8b) in rats exposed to  $\frac{1}{2}$ the WHO-recommended dose of CYN and lowered to 0.140  $\mu$ **g**.kg<sup>-1</sup> of CYN well.

> The predominant lesions were observed in the outer medulla, and cortical tubular changes in rat groups exposed to different concentrations of MC-LR:  $0.105 \mu g.kg^{-1}$ ,  $0.070$  $\mu$ g.kg<sup>-1</sup>, 0.035  $\mu$ g.kg<sup>-1</sup>, 0.091  $\mu$ g.kg<sup>-1</sup>. Significant renal changes in the  $0.105 \mu g \text{ kg}^{-1}$  exposure group included



Fig. 5: Haematoxylin and eosin stain of Histopathological features in kidney sections after 90 days of exposure to 0.175 µg.kg-1 of CYN Fig. 5a Fig. 5d Cellular swelling (yellow arrow) with luminal protein (blue arrow) ×400, Fig. 5e Glomerular collapse (yellow arrow) ×100. Control ×100, Fig. 5b 0.175 µg.kg-1 of CYN – Nuclear pyknosis (yellow arrows) ×400, Fig. 5c Pigmentation – Tubular cells (yellow arrow) ×400,



Fig. 6: Haematoxylin and eosin stains of Histopathological features in kidney sections after 90 days of exposure to CYN at 0.161 µg.kg<sup>-1</sup>. Fig. 6a Control ×100, Fig. 6b 0.161  $\mu$ g.kg<sup>-1</sup> – Cellular swelling (yellow arrow) and inflammation (blue arrow) ×400, Fig. 6c CYN at 0.161  $\mu$ g.kg<sup>-1</sup> - Tubular epithelial swelling (yellow arrow) and vascular congestion (blue arrow) ×400.





Fig. 7: Haematoxylin and eosin stains of histopathological features in kidney sections after 90 days of exposure to the CYN at 0.140 µg.kg<sup>-1</sup>. Fig. 7a Control  $\times$ 100, Fig. 7b CYN at 0.140 µg.kg<sup>-1</sup> – Cellular swelling of renal tubules (yellow arrow)  $\times$ 400.



Fig. 8: Haematoxylin and eosin stains of histopathological features in kidney sections after 90 days of exposure to CYN at 0.105  $\mu$ g.kg<sup>-1</sup>. Fig. 8a Control  $\times$ 100, Fig. 8b CYN at 0.105 µg.kg<sup>-1</sup> – Cellular swelling of renal tubules (yellow arrow)  $\times$ 400.

 $(Fig. 9c)$ , vascular congestion  $(Fig. 9e)$ , and mild interstitial luminal protein (Fig. 9b), tubular epithelial swelling inflammation (Fig. 9d). Mild tubular epithelial swelling (Fig. 10b) and luminal proteins (Fig. 10c) were observed cellular swelling (Fig. 11b) and luminal proteins (Fig. Mild epithelial swelling (Fig. 12b) was significant in the  $0.035 \mu$ g.kg<sup>-1</sup> exposure group.

and interstitial inflammation were mild to severe records<br>including epithelia swelling and intraluminal protein. Thus the luminal proteins (Fig. 10c) were observed to be significant of the second vertex of the significant in  $\frac{1}{2}$  exposure group. The second in  $\frac{1}{2}$  exposure group. The significant in the significant in  $\frac{1}{2}$  expo The predominant changes observed histologically were confined to the renal tubules in both CYN and MC-LR exposed groups. Luminal proteins, nuclear pyknosis, cellular swelling, luminal protein, pigmentation, vascular congestion, in 0.175  $\mu$ g.kg<sup>-1</sup> and 0.161  $\mu$ g.kg<sup>-1</sup> doses of CYN-treated groups. Since the tubular epithelium plays a critical role in homeostasis, damage to tubules is expected to impair renal functions (Liu et al. 2018). Further, the mild pyknotic nuclei that occurred in cortex tubules may indicate an early stage

inal protein (Fig. 9b), tubular epithelial swelling of renal damage in 0.175  $\mu$ g.kg<sup>-1</sup> doses of CYN-treated The predominant reference of the outer were observed in the changes were most marked in the group led with a to be significant in 0.091  $\mu$ g.kg<sup>-1</sup> exposure group. Mild higher dose than the 0.140  $\mu$ g.kg<sup>-1</sup> and 0.161 groups. The histological changes in the current study confirm cyanotoxin-induced renal damage due to tubular pathology, and the changes were most marked in the group fed with a higher dose than the  $0.140 \mu$ g.kg<sup>-1</sup> and  $0.161 \mu$ g.kg<sup>-1</sup> group.

11c) were significant in the 0.070  $\mu$ g.kg<sup>-1</sup> exposure group. Although CYN is often defined as a hepato-toxin, Chernoff  $\frac{1}{2}$  ug.kg exposure group.<br>investigated CYN-initiated dose-dependent renal toxicity in luminal proteins in both CTIV and MC-LIV tubules with increasing concentration of CYN was recorded<br>Seed groups. Luminal proteins, nuclear pyknosis, cellular Although CYN is often defined as a hepato-toxin, Chernoff et al. (2018) described that the kidney was the most sensitive organ for CYN exposure in male mice. Chernoff et al. (2018) both cortex and medulla. An increase in lesions in cortical in the study. In the renal cortex, microscopic changes in this region consisted of epithelial cytoplasmic alterations, including epithelia swelling and intraluminal protein. Thus, the results of the present study support the findings and agreement

occurred in cortex tubules may indicate an early stage swelling, vascular congestion and interstitial inflammation The presence of mild luminal protein, tubular epithelial



Fig. 9: Haematoxylin and eosin stain of Histopathological features in kidney sections at 90 days of exposure to 0.105 µg.kg<sup>-1</sup> of MC-LR. Fig. 9a (yellow arrow)  $\times$ 400, Fig. 9d 0.105  $\mu$ g.kg<sup>-1</sup> of MC-LR - Mild interstitial inflammation (yellow arrow)  $\times$ 400, Fig. 9e 0.105  $\mu$ g.kg<sup>-1</sup> of MC-LR - Vascular Control ×100, Fig. 9b 0.105 µg.kg<sup>-1</sup> of MC-LR – Luminal protein (yellow arrow) ×400, Fig. 9c 0.105 µg.kg<sup>-1</sup> of MC-LR - Tubular epithelial swelling congestion (yellow arrow) ×400.



Control ×100, Fig. 10b MC-LR at 0.091 µg.kg<sup>-1</sup> -Tubular epithelial swelling (yellow arrow) ×400, Fig. 10c MC-LR at 0.091 µg.kg<sup>-1</sup> - Intraluminal Fig. 10: Haematoxylin and eosin stains of histopathological features in kidney sections at 90 days of exposure to MC-LR at 0.091 µg.kg<sup>-1</sup>. Fig. 10a protein (yellow arrow) ×400.





Control ×100, Fig. 11b MC-LR at 0.070  $\mu$ g.kg<sup>-1</sup> – Tubular epithelial swelling (yellow arrow) ×400, Fig. 11c MC-LR at 0.070  $\mu$ g.kg<sup>-1</sup> – Intraluminal Fig. 11: Haematoxylin and eosin stains of Histopathological features in kidney sections after 90 days of exposure to MC-LR at 0.070 µg.kg<sup>-1</sup>. Fig. 11a protein (yellow arrow) ×400.



Fig. 12: Haematoxylin and eosin stains of Histopathological features in kidney sections at 90 days of exposure to MC-LR at 0.035 µg.kg<sup>-1</sup>. Fig. 12a  $M_{\rm eff}$  at 0.035  $\mu$  mc-late  $\mu$  1. Fig. 12b McC-LR at 0.035  $\mu$ Control  $\times$ 100, Fig. 12b MC-LR at 0.035 µg.kg<sup>-1</sup> – Tubular epithelial swelling (yellow arrow)  $\times$  400.

significant structural changes in the kidney in mice exposed in rats' exposure to  $1\mu g$ .L<sup>-1</sup> of MC-LR (0.070  $\mu g$ .kg<sup>-1</sup>). Further, with the increase in exposure concen LR for 3 months enlarged renal corpuscles with compressed in the renal pelvis has been recorded (Yi et al. 2019 groups, no significant lesions were observed in the glomeruli more lymphocytes than the 3-month groups (Yi et al. was recorded in MC-LR exposed rats in this study. No to 1  $\mu$ g.L<sup>-1</sup> and 30  $\mu$ g.L<sup>-1</sup> MC-LR were observed (Yi et al. 2019). In the present study, intraluminal proteins and mild epithelial swelling were significant in cortex tubules Nevertheless, it was reported that exposure to  $60 \mu g.L^{-1}MC$ Bowman's space (Yi et al. 2019). In all MC-LR exposure of rat kidneys in the present study. Exposure to MC-LR for 6 months, obvious lymphocyte infiltrate in interstitial tissue was significant in mice treated with 60  $\mu$ g.L<sup>-1</sup> MC-LR, with

). In the present study, intraluminal proteins and tubules were enlarged and filled with eosinophilic material dilated renal tubule. Moreover, exposure to 90  $\mu$ g. $L^{-1}$  and  $120 \mu$ g. $L^{-1}$  MC-LR, numerous renal corpuscles enlarged with compressed Bowman's space in the kidney cortex, and renal recorded (Yi et al. 2019).

s, obvious lymphocyte infiltrate in interstitial tissue chronic exposure to CYN and MC-LR is associated with Further, with the increase in exposure concentration of MC-LR in mice, an increase in lymphocyte infiltration in the renal pelvis has been recorded (Yi et al. 2019). The mice that were exposed to MC-LR for 6 months displayed more lymphocytes than the 3-month groups (Yi et al. 2019). The results of the present study indicate the possibility that nephrotoxicity. We demonstrated that chronic oral exposure

to CYN and MC-LR leads to nephrotoxicity in Wistar rats, as evidenced by changes in concentrations of serum creatinine, urine creatinine, and urinary biomarker KIM-1. Most importantly, the rats exposed to the environmental samples contaminated with CYN and MC-LR exhibited renal toxicity. Therefore, it could be debated that prolonged exposure to CYN and MC-LR may lead to chronic kidney disease.

### **CONCLUSION**

The concentrations of CYN and MC-LR at and above the recommended WHO levels and environmental samples used in this study induced signs of mild to moderate renal injury in Wistar rats as evidenced by elevated serum creatinine values, urinary KIM-1, and mild to moderate renal pathological changes consistent with tubular interstitial involvement. These results provide significant evidence that consumption of CYN and MC-LR-contaminated drinking water could lead to kidney injury, possibly leading to CKD.

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