Beni-Suef University Journal of Basic and Applied Sciences 6 (2017) 69–75

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Beni-Suef University
Journal of Basic and Applied Sciences

journal homepage: www.elsevier.com/locate/bjbas

Full Length Article

Oxidative stress biomarkers in *Clarias gariepinus* (Burchel, 1822) exposed to Microcystin-LR

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ARTICLE INFO

Article history:
Received 20 December 2016
Received in revised form 17 January 2017
Accepted 19 January 2017
Available online 20 January 2017

Keywords:
Microcystin-LR
Microcystis aeruginosa
Oxidative stress
Hepatotoxin
Clarias gariepinus

ABSTRACT

Stress levels inflicted by microcystin-LR extracted from *Microcystis aeruginosa* were assessed by estimating the effects on the oxidative stress biomarkers in the liver, muscle and gill tissues of *Clarias gariepinus*. Microcystin-LR was administered to the fish by injection into their intra-peritoneal cavity. Microcystin-LR induced the activities of mainly Glutathione-S-Transferase (GST), Catalase (CAT) and Superoxide dismutase (SOD) in the liver on dose and temporal basis, while it inhibited the activities of Cytochrome P450 on temporal basis in the liver and gill. Results show that MC-LR significantly induced oxidative stress in the *C. gariepinus*. Findings show that 400 μg MC-LR/kg b.w. disrupted the antioxidant status of the fish without any external physical manifestation on the body. Microcystin-LR induced the activities of mainly GST, CAT and SOD in the liver on dose and temporal basis while it inhibited the activities of CYP450 on temporal basis in the liver and gill. The adopted MC-LR doses; particularly 400 μgMC-LR/kg b.w. inflicted stress on the fish without any external/physical manifestation. MC-LR impacted on the antioxidant enzymes in the fish; in the order of liver > gill > muscle. This research has provided empirical evidences that 400 μg MC-LR/kg b.w induced oxidative stress in *C. gariepinus* in less than a month of exposure. This adopted level of MC-LR can be used as a warning signal in natural water bodies and commercial *C. gariepinus* ponds.

1. Introduction

Eutrophic aquatic ecosystems enhance the proliferation of cyanobacteria blooms particularly in warm conditions. These cyanobacteria; also known as blue-green algae contain cells which produce biotoxins e.g. Microcystins; which cause harms to aquatic biota and vertebrates generally (Yoo et al., 1995). The ecological concern of this study stems from the fact that cyanobacteria have been documented to be palatable to innumerable fish species (Bowen, 1982). It is noteworthy that high numbers of toxic *Microcystis* cells have been recorded in the intestine of tilapia (*Oreochromis niloticus*). This is an indication that the fish feeds on toxic cyanobacteria (Mohamed et al., 2003). Some literatures have also described cyanobacteria as an important component of tropical cichlids and cyprinids diet (Bowen, 1982; Beveridge et al., 1993). Some experimental findings have proven that animals prefer water containing strains of cyanobacteria to clean water (Lopez and Costas, 1999; Stewart et al., 2008). Albeit cyanobacterial species abound, the species associated with microcystin production is *Microcystis aeruginosa.*

When *Microcystis aeruginosa* dies, the cell walls burst, releasing the microcystin into the water. Microcystins are very stable; hence resist chemical breakdowns such as hydrolysis, oxidation and reduction under normal atmospheric conditions. At higher temperatures i.e. >40 °C and extreme pH of <1 or >9, microcytins may break down, but rather very slowly. However, the persistence of microcystins at >100 °C was reported by WHO (1999). This is of great health concern because water treatment by boiling does not guarantee absence of the persistent biotoxin. However, microcystins breaks down slowly in full sunlight especially when water-soluble pigments are present (Tsuji et al., 1995). Although microcystins can be broken down by some bacterial proteases, but in real case scenarios the proliferation of cyanobacteria often exceeds that of the bacteria in natural water bodies, hence the toxin persists for long periods of time, causing ecological and health devastations.

Of all Microcystin congeners, Microcystin- LR (MC-LR) is the most toxic and most abundant (OEHHA, 2009). Hence, attention of the current study shall be focused on the toxicity of MC-LR. The IUPAC name of Microcystin- LR ($C_{49}H_{47}N_{10}O_{12}$) is (5R, 8S, 11R, 12S, 19S, 22R)-15-[3-(diaminoethylidenemino) propyl]-18[(1 E,3E,5S,6S)-6-Methoxy-3,5-dimethyl-7-phenylhepta-1, 3-dienyl]-1,5,12,19-tetramethyl-2-methylidene-8-(2-methylpropyle) 3,6,9,13,16,20,25-hepatox-1,4,7,10,14,17,21-hetazacyclopentacosane-11

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http://dx.doi.org/10.1016/j.bjbas.2017.01.005
2314-8535/

,22-dicarboxylic acid. In a short form, it can also be called 5-L-Arginine-microcystin LA. The Molar mass of MC-LR is 995.19 $g{\cdot}mol^{-1}$, Density is 1.299 g/cm^3 and its solubility in ethanol is 1 mg/mL. Microcystin-LR prevents protein phosphatase from functioning through non-competitive inhibition by its 3-amino-9-methoxy-2, 6, 8-trimethyl-10-phenyl-4, 6-decadienoic acid (ADDA) group.

Extensive ecological studies have been carried out to investigate the fate and deterministic modes of action of Microcystin-LR when released into the environment. Sequel to its mode of action, MC-LR can be classified as a hepatotoxin. Due to the fact that it is a water-borne toxicant, its occurrence has been associated with serious public health concerns (Carmichael, 1992). It is noteworthy that the highest incidence of liver cancer is endemic in areas with rivers in which appreciable cyanobacteria have been detected (Fawell et al., 1993). Albeit some studies suggest that MC-LR might act as tumor promoters i.e. as agents that do not cause cancer, but stimulate the proliferation of cancer cells (IARC, 2006). One major toxigenic mechanisms is the inhibition of protein phosphatase 1 and 2A which culminates in increased protein phosphorylation, ultimately resulting in necrosis and compromised liver architectural integrity and function (Malbrouck and Kestemont, 2006), cytoskeletal rearrangement, apoptosis and intrahepatic hemorrhage (Fischer and Dietrich 2000; Fischer et al., 2000). This is the basis of MC-LR cytotoxic effects and tumor-promoting activity (Hooser et al., 1989; Carmichael, 1994; Hooser, 2000). More so, oxidative stress also plays a significant role in the pathogenesis of MC-LR toxicity in vertebrates (Ding et al., 2001; Towner et al., 2002; Ding and Ong, 2003; Moreno et al., 2003; Zegura et al., 2004). However, some studies have demonstrated high toxicity of MC-LR to other organs than the liver. For example, Carps exposed to MC-LR elicited sever damage to renal proximal tubular cells and hepatocytes (Fisher and Dietrich, 2000). Damages to other organs i.e. heart (Best et al., 2001), bones (Bury et al. (1995) etc.; have also been recorded.

There is dearth information on oxidative stress of MC-LR exposed catfish probably due to paucity of research interest in this aspect. However, regardless of species, the mechanism of action of MC-LR is the same (OEHHA, 2009). Enormous research efforts have been made on toxicity of MC-LR on livestock animals such as birds and dogs. Takahashi and Kaya (1993) observed histopathological lesions in the liver of quail injected intra-peritoneally with 256 μg microcystin/ kg body weight. Bird deaths have been traced to cyanobacterial blooms in Canada and the United States since the early 1900 s (Landsberg, 2002). Death toll of birds in Canada and United States; sequel to microcystin poisoning was estimated at a range of a few individuals to several thousand birds per incident (OEHHA, 2009). In California, high mortality in birds wintering at the Salton Sea was attributed to MC-LR (Carmichael and Li, 2006). Furthermore, in many parts of North America; such as California, Colorado, Georgia, Michigan, Mississippi, Oklahoma, Wisconsin, and even as far as Saskatchewan in Canada, devastating domestic animal poisonings have been scientifically attributed to blooms of *Microcystis sp.* (OEHHA, 2009). Elsewhere in Caruaru, Brazil; sometime in February 1996 MC-LR was detected in the blood and liver of 116 patients who suffered from visual disturbances, nausea, vomiting, and muscle weakness. 100 of them suffered acute liver failure and 52 eventually died (Azevedo et al., 2002). Compared to other animals, fish are at a relatively higher risk of exposure to MC-LR due to the fact that they are at the top of the aquatic food chain, hence have greater tendencies of accumulating the biotoxin from organisms at lower trophic levels in conjunction with the process of bioaccumulation from the aqueous phase through ingestion and respiration.

Oxidative stress biological markers are important tools which can be employed in MC-LR toxicity assessment in fish. Liver (hepatopancreas) plays a key role in xenobiotic detoxication in fish. Polarity of a xenobiotic increases within two phases of metabolization through oxidation, reduction and hydrolysis reactions and subsequently the produced metabolite is conjugated with an endogenous substrate and excreted through the basolateral membrane. This implies that activity and concentrations of enzymes and substrates involved in the detoxification processes of MC-LR can be quantified by assessing the rates of its induction and inhibition of oxidative stress enzymes.

Previous studies have established the fact that intra-peritoneal (i.p.) injection experiments elicit higher toxicity in subjected species than dietary and aqueous exposures (OEHHA, 2009). Therefore, i.p. administration being of higher toxicity, hence detectability guarantees safer decisions. This study was aimed at administering microcystin-LR to *Clarias gariepinus* intraperitoneally; employing oxidative stress biomarkers in the liver, muscle and gill tissues as toxicity assessment tools.

2. Material and methods

A static bioassay was conducted for 28 days using *Clarias gariepinus* as test organisms. This was carried out with a view to studying the numerical estimations of induction or inhibition of MC-LR on selected oxidative enzymes in the fish. 200 and 400 μg/ kg MC-LR and intraperitoneal administration were chosen with reference to several previous studies. The choice of fish (*Clarias gariepinus*) was informed by its ability to accumulate toxicants in its adipose tissue (Isibor et al., 2016), its ability to withstand stress and its high commercial value in Nigeria. *C. gariepinus* of average weight of 120 ± 1.2 g and average standard length of 30.5 ± 2.1 cm were obtained from a fish farm in Benin City, Edo State, Nigeria. The choice of farm was informed by prior screening for cyanobacteria and other pollutants which might be potential stressors to the fish in the pond.

2.1. Acclimatization of Clarias gariepinus

Firstly, all selected fish were inspected for disease conditions and general fitness. 24 selected viable individuals were acclimatized for 28 days under natural day and night photoperiods (12/12-h) prior to the commencement of the toxicity bioassay in 3 (8 fish in each) glass aquaria ($60 \times 48 \times 48$) cm^3 containing constantly aerated 96 L of non-chlorinated freshwater. The fish were allowed to acclimatize for 15 days under laboratory conditions to allow adaptation to experimental conditions. The fish were fed during the period of acclimatization with fish feed and the water was replaced every 24 h. The physico–chemical parameters of the water used were examined. These parameters included temperature which was maintained at 27 ± 2 °C, dissolved oxygen was 7.5 ± 0.6 mg/L and the hydrogen ion concentration (pH = 8). The temperature and the dissolved oxygen of the water were measured on daily basis with a Model JPSJ-605 DO-Analyzer, while the pH was measured using the Electric Probe Hydro-lab water quality meter (HANNA HI 9813 GRO).

2.2. Microcystis aeruginosa collection and MC-LR extraction

With the aid of a plankton net (20 μm mesh size) algal samples were collected from the surface of an algae-invaded section of Jemaison River in Delta State, Nigeria. 250 ml dark bottles with lids were properly rinsed with distilled water and the algal samples were collected in them and kept in a cooler. They were then transported immediately to the laboratory for analysis.

In the laboratory, culture of the algal samples were centrifuged in the 250 mL bottles; in batches at 3500 rpm for 2 h to

concentrate the algal cells into a pellet. The cell pellet was then lyophilized for 48 h using a freeze-dry system [35,49]. The lyophilized cells were extracted three times with 10 mL 0.1 M acetic acid and 20 mL of a mixture of methanol: chloroform (1:1 v/v). The cell suspension was sonicated in an ultrasound bath for 15 min, stirred for 30 min at room temperature, and then centrifuged at 4500 rpm for 15 min. Only MC-LR was identified in the cyanobacterial cell extracts analyzed using a Varian 9012 equipped with a Varian ProStar 330 Diode Array Detector. Microcystin congeners were identified by their UV spectra and by using commercial microcystin-LR, MC-RR and MC-YR standards provided by Beacon Analytical System Inc.in Microcystin Plate Kit; Cat. No. 20-0068. MC-LR constituted 92% (6800 μg/kg dry weight) of the total microcystins observed.

2.3. Administration of MC-LR to Clarias gariepinus

Feeding was discontinued on commencement of the experimental regime. Three experimental set-ups; each comprising of 8 individuals of *C. gariepinus* were employed in the experiment. Concentration of MC-LR administered to each experimental fish was calculated using the weight i.e. MC-LR concentration = TC X W

Where TC = target concentration i.e. 200 or 400 μg/L, and W = weight of each individual. The derived concentration was dissolved in 1 ml of water (vehicular medium).

The control experiments were injected with 1 ml of water and the second set were injected with 200 μg/kg body weight, while the third set were injected with 400 μg/kg body weight. Four individuals were collected from each aquarium after 14 days and the remaining 4 were collected after 28 days. They were immediately dissected with sterile surgical blade and syringe; to collect their liver, gills and muscles. The samples were preserved in a freezer till further analysis.

2.4. Analysis of antioxidant enzymes

The thawed gill, muscle and liver samples were separately homogenized in buffer (pH 7.4), centrifuged at 10,000*g* for 20 min at 4 °C. The supernatant was further centrifuged at same 100,000*g* for 1 h at 4 °C. The final supernatant was drained and the pellet was washed and preserved in the buffer (pH 7.4); as illustrated by Siroka et al. (2005). Each suspension was then put into an Eppendorf tube and stored at −85 °C pending enzymatic assays.

Catalase (CAT) activity was assayed by the procedures demonstrated by Beers and Sizer (1952). Spectrophotometer was used to read the reduction of hydrogen peroxide (H_2O_2) at 240 nm; using 1.0 mL quartz cuvettes with a light path of 1.0 cm. CAT levels were expressed in nmol H_2O_2 consumed/min/mg protein.

Superoxide dismutase (SOD) activity was measured using the xanthine oxidase–cytochrome method as described by McCord and Frodovich (1969). Xanthine reacted with 2-[4-iodophenyl]-3-[4-nitrophenyl]-5-phenyltetrazolium chloride to form superoxide radicals which reacted to form a red coloured formazan. The red formazan was used to determine the SOD activity in the tissues i.e. presence of SOD readily bound with superoxide radicals, thereby reducing the availability of superoxide radicals, ultimately inhibiting formation of formazan. SOD activity was measured spectrophotometrically at 505 nm and calculated as inhibition percent of formazan formation. The concentration of Cytochrome P450 (CP450) was quantified spectrophotometrically at 400–490 nm, on the basis of the difference between absorbance readings at 450 nm. Glutathione-S-Transferase (GST) activities in the tissues samples were analyzed by extracting the tissues separately with a phosphate buffer (pH 7.2), homogenized and centrifuged at 10,500*g* for 20 min at 4 °C. The activity of GST was investigated in supernatants spectrophotometrically according to the recommendation of Habig et al. (1974).

2.5. Statistical analysis

Results were presented as the mean ± S.E. The differences between the data from the different concentrations of MC-LR administration for 14 and 28 days were analyzed by one-way analysis of variance (ANOVA). Duncan Multiple Range test (DMR) was used to ascertain the actual locations of the significant differences at $P < 0.05$; using the MS Excel Statistical Tool Package (version 19).

3. Results and discussion

As earlier stated, it is note-worthy that the ADDA group is the major active component in MC-LR (see Fig. 1). Results show significant responses of the fish to Microcystin-LR at different concentrations and time durations. No fish mortality was recorded throughout the experimental regime. Visual observation showed a progressive sluggishness in the set of fish injected with 400 μg MC-LR/kg body weight; which became most apparent after 26 days. The behavior of the individuals injected with 200 μg MC-LR/kg body weight was not different from that of the control throughout the period of the experiment. On the whole, no changes were observed externally on all the sets of the experiment throughout the study.

3.1. Effect of MC-LR on activity of Cytochrome P450 (CYP450)

Microcystin-LR concentrations used in the experiment elicited significant effects in the levels of CYP450 (Fig. 2). The activities of CYP450 in the fish individuals of both experimental set-ups (200 and 400 μg/kg b.w.) were significantly higher after 14 days than 28 days ($P < 0.05$). Generally, there was a sharp rise followed by an abrupt drop in CYP450 activities in catfish exposed to 400 μg/kg b.w. after 28 days; back to a level of no significant difference from the control ($P > 0.05$). In the process of detoxification of MC-LR through oxidation process, CYP450 is converted to CYP420; thereby reducing the level of the former. This trend is a bit similar to the reduction of CYP450 observed in mice exposed to 125 μg/kg (Brooks and Codd, 1987).

Temporally heterogenous levels of CYP450 were observed in the gills in both experimental doses i.e. the activity levels of CYP450 were significantly higher in the cases of 14 days than the 28 days ($P < 0.05$). This result also demonstrates some responsiveness of the enzyme to the presence of the biotoxin in the gills. After 28 days, the levels of CYP450 in both experimental cases were no longer different from that of the control (Fig. 3).

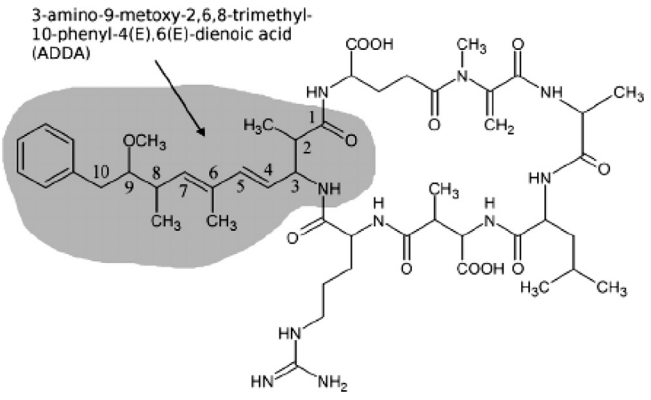

Fig. 1. Molecular structure of Microcytine-LR.

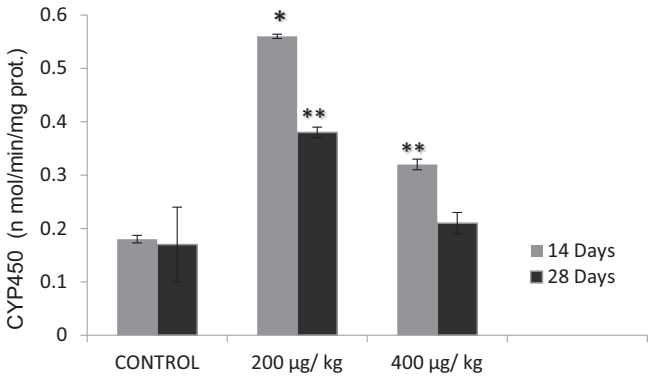

Fig. 2. Cytochrome P450 activity level in the Liver of *C. gariepinus* exposed to different doses of MC-LR for 14 days and 28 days. *Signifies significant difference at P < 0.05. Sample replicate N = 4.

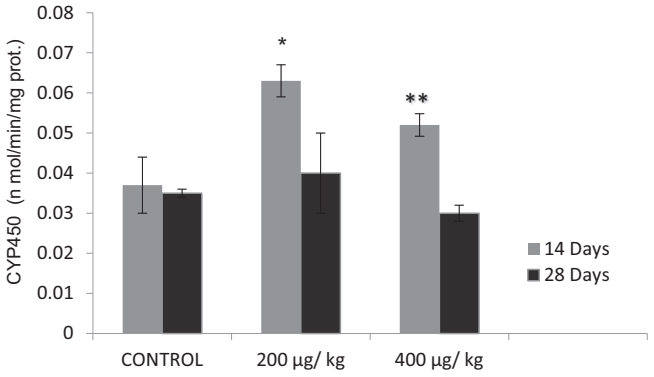

Fig. 3. Cytochrome P450 activity level in the gill of *C. gariepinus* exposed to different doses of MC-LR for 14 days and 28 days. *Signifies significant difference at P < 0.05. Sample replicate N = 4.

There was no significant difference between the levels of CYP450 in the muscle in both experimental cases and that of the control (P > 0.05). This is an indication that the MC-LR must have had minimal impacts on the gill tissues of the fish (Fig. 4). Albeit aqueous exposure may show significant CYP450 activity levels in the gill due to the fact that the gill is the first receptor organ of toxicants in the real scenario i.e. it is in direct contact with the toxicants in the ambience.

The activities of CYP450 in the experimental set-ups were however highest in the liver compared to the gill and muscle i.e. the activity level of CYP450 in the liver ranged from 0.23–0.55 nmol/min/mg protein, while the highest values recorded

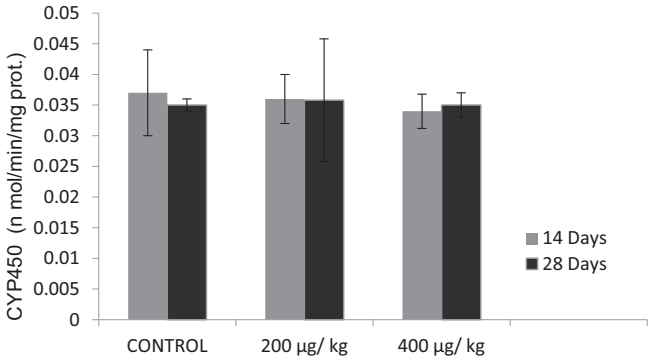

Fig. 4. Cytochrome P450 activity level in the muscle of *C. gariepinus* exposed to different doses of MC-LR for 14 days and 28 days. Sample replicate N = 4.

for gill and muscle were 0.063 and 0.035 nmol/min/mg protein respectively. Induction of CYP450 activity in the liver of mice as a result of exposure to toxicants was earlier documented by Bleeke et al. (1985). Current results have buttressed previous findings that MC-LR may elicit hepatotoxicity in humans (OEHHA, 2009).

3.2. Effects of MC-LR on activity of Glutathione-S-Transferase (GST)

The activity levels of GST in the liver of the fish subjected to 400 µg/kg b.w. were very much significantly higher than that of 200 µg/kg b.w. (P < 0.05); which was also significantly higher than the control experiment (Fig. 5). While no significant differences were observed in the gill and muscle (Figs. 6 and 7 respectively). This implies that 400, followed by 200 µg MC-LR/kg b.w. had significant impact on the liver tissue. The level of GST activity is quite significant in the liver because GST is a major enzyme required in the metabolism of MC-LR; through conjugation in the liver (Kato et al., 2001; George, 1994; Balendiran et al., 2004). GST also serves as a substrate for other antioxidant enzymes and protects the cells through non-enzymatic scavenging of free radicals (Kovarova and Svobodova, 2009).

Results show that GST appears to be the most promising biomarker in terms of proportionate response to concentration of MC-LR and duration of exposure. Induction of GST activity in the liver of *Gasterosteus aculeatus* in response to prochloraz was earlier demonstrated by Sanchez et al. (2008).

3.3. Effects of MC-LR on activity of Catalase (CAT)

The activity level of CAT in the liver shows significant concentration and temporal heterogeneity. The activity levels of CAT in the liver of both experiments were significantly higher on the 28th day than the 14th day (P < 0.05). Jos et al. (2005) observed that oral dose of 60 µg MC-LR/fish/day induced CAT activity of 780 nmol/min/mg protein in the liver of male *Oreochromis sp.* after 21 days. *C. gariepinus* of the current study; exposed to 400 µg MC-LR/kg b.w. exhibited only a little higher oxidative stress than *Oreochromis sp.* exposed to 60 µg MC-LR/fish/day. This implies that *C. gariepinus* has a relatively high resilience and can withstand higher level of stress.

The level of CAT activity was very much higher in the liver than other tissues which showed no significant CAT activity at different concentrations and time duration. This is an indication of higher impact of MC-LR on the liver than the gill (Fig. 9) and muscle tissues (Fig. 10).

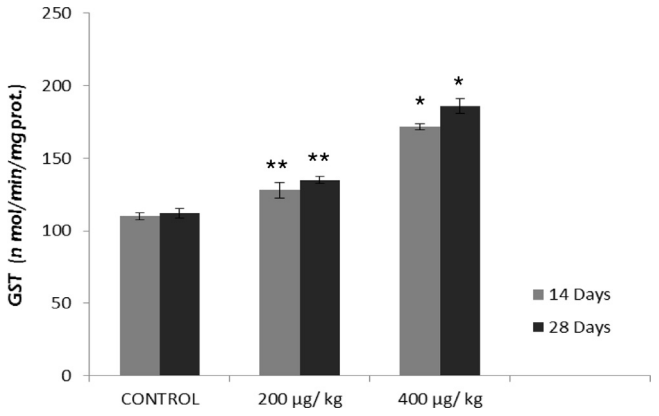

Fig. 5. GST activity level in the liver of *C. gariepinus* exposed to different doses of MC-LR for 14 days and 28 days. *Signifies significant difference at P < 0.05. Sample replicate N = 4.

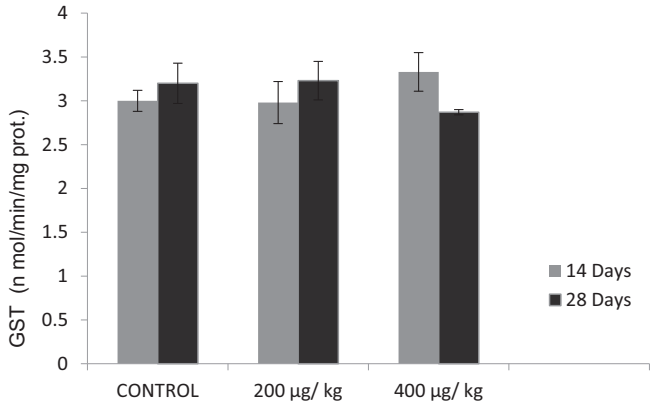

Fig. 6. GST activity level in the gill of *C. gariepinus* exposed to different doses of MC-LR for 14 days and 28 days. Sample replicate N = 4.

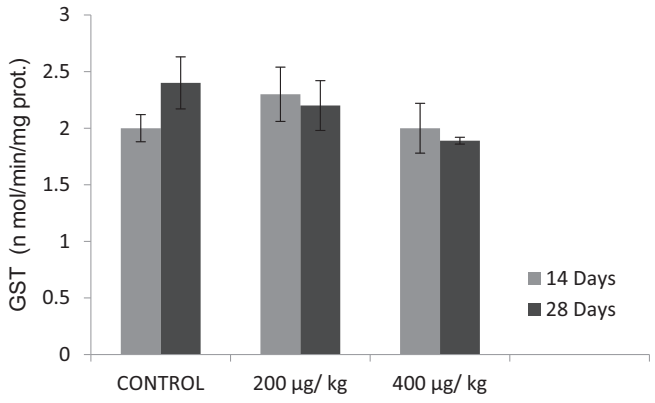

Fig. 7. GST activity level in the muscle of *C. gariepinus* exposed to different doses of MC-LR for 14 days and 28 days. Sample replicate N = 4.

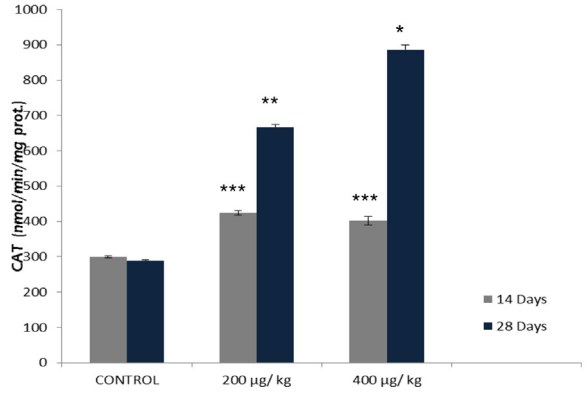

Fig. 8. Catalase (CAT) activity level in the liver of *C. gariepinus* exposed to different doses of MC-LR for 14 days and 28 days. *Signifies significant difference at $P < 0.05$. Sample replicate N = 4.

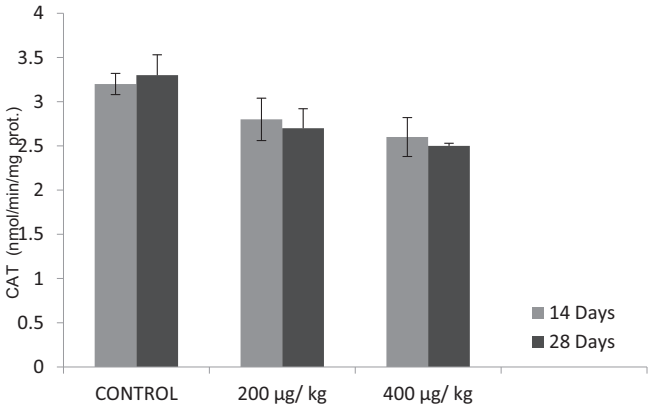

Fig. 9. Catalase (CAT) activity level in the gill of *C. gariepinus* exposed to different doses of MC-LR for 14 days and 28 days. Sample replicate N = 4.

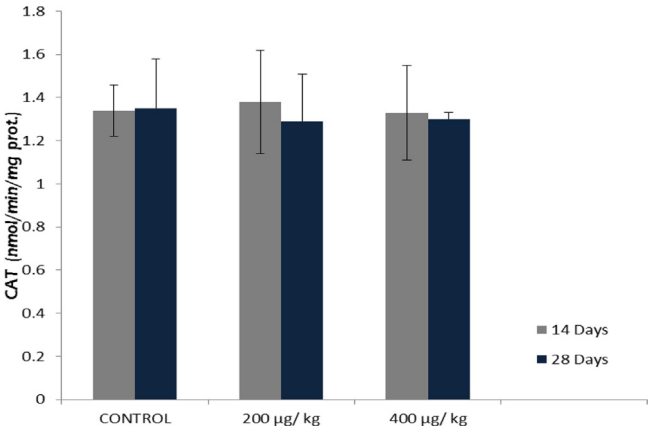

Fig. 10. Catalase (CAT) activity level in the muscle of *C. gariepinus* exposed to different doses of MC-LR for 14 days and 28 days. Sample replicate N = 4.

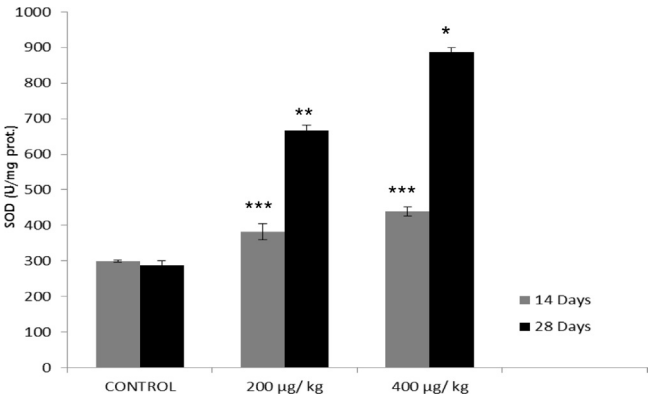

Fig. 11. Superoxide dismutase (SOD) activity level in the liver of *C. gariepinus* exposed to different doses of MC-LR for 14 days and 28 days. *Signifies significant difference at $P < 0.05$. Sample replicate N = 4.

3.4. Effects of MC-LR on activity of Superoxide dismutase (SOD)

There was a simultaneous induction response in CAT (Fig. 8) and SOD (Fig. 11) activity in the liver of the fish. This conforms to the expected trend according to the demonstration of Dimitrova et al. (1994). This is as a result of close similarity in their biochemical functions in metabolism of the toxicant.

In conformity to other oxidative enzymes; there was no significant difference in the activity levels of SOD in the gill and muscle among all experimental set ups. This further buttresses the fact that MC-LR had no significant impacts in the gill (Fig. 12) and the muscle (Fig. 13) of the fish.

No discernible pattern was observed in the SOD activity in the muscle of the experimental fish in both doses and periods. This also implies that MC-LR had no significant impact on the muscle tissue.

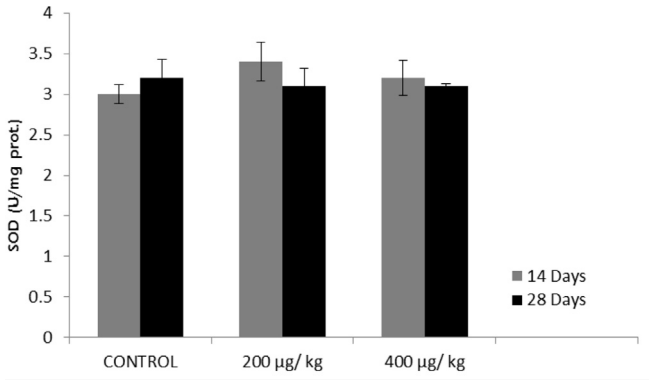

Fig. 12. Superoxide dismutase (SOD) activity level in the gill of *C. gariepinus* exposed to different doses of MC-LR for 14 days and 28 days. Sample replicate N = 4.

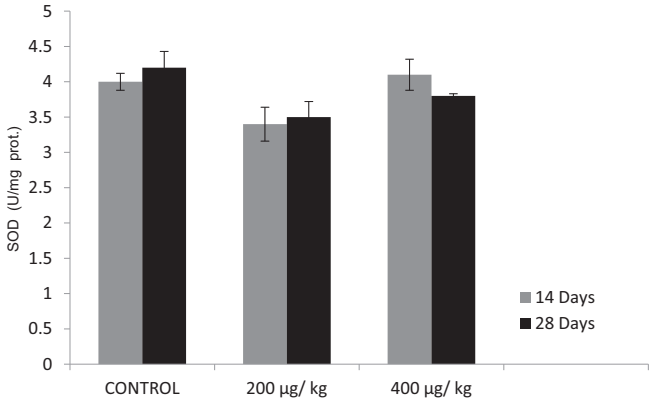

Fig. 13. Superoxide dismutase (SOD) activity level in the muscle of *C. gariepinus* exposed to different doses of MC-LR for 14 days and 28 days. Sample replicate N = 4.

On the whole, spatial heterogeneity was observed in all the oxidative stress enzymes; showing that MC-LR toxicity varied across the tissues in the order of liver > gill > muscle. This is in conformity to the findings of Jos et al. (2005). With the findings that 400 µg MC-LR/kg b.w. is mainly of impact in the liver of the fish, it is somewhat logical to advise removal of the liver of the fish from affected water bodies before consumption to prevent ingestion of the biotoxin by consumers. This idea is however negated by the fact that shellfishes e.g. periwinkles, crabs, shrimps etc.; which are potential accumulators of MC-LR are consumed wholly. Temporally heterogeneous activities of all the enzymes (except GST) was also observed; particularly in the fish injected with 400 µg MC-LR/kg b.w. This implies that 400 µg/kg b.w. elicited significant oxidative stress in the liver of the *Clarias gariepinus* after 28 days. However, previous reports show that most affected lakes have recorded higher concentrations than the levels demonstrated in this study (OEHHA, 2009). Extrapolations from the current results necessitate a stringent and prompt remedial responses in events of algal bloom.

4. Conclusion

Microcystin-LR induced the activities of mainly GST, CAT and SOD in the liver on dose and temporal basis while it inhibited the activities of CYP450 on temporal basis in the liver and gill. Results show that MC-LR significantly induced oxidative stress in the *C. gariepinus*. The findings conform to the fact that MC-LR is a hepatotoxin (OEHHA, 2009). The adopted MC-LR doses; particularly 400 µgMC-LR/kg b.w. inflicted stress on the fish without any external/ physical manifestation. MC-LR impacted on the antioxidant enzymes in the fish; in the order of liver > gill > muscle. This research has provided empirical evidences that 400 µg MC-LR/kg b.w induced oxidative stress in *C. gariepinus* in less than a month of exposure. This adopted level of MC-LR can be used as a warning signal in natural water bodies and commercial *C. gariepinus* ponds.

Competing interests

No competing interests exist.

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