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Research Article

IMPACTS OF MICROCYSTIN TOXIN ON ANTIOXIDANTS AND LIPID PEROXIDATION MARKERS IN COMMON FISH *CYPRINUS CARPIO* DURING CHRONIC DURATION

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Abstract

The aim of presents study revealed the effects of purified Microcystin toxin from *anabaena circinalis* on the levels of antioxidants and lipid peroxidation markers during chronic duration in common carp, the statistical analysis appeared significant differences between all antioxidants at the P0.05 level and lipid peroxidation markers in intraperitoneal common carp and control.superoxide dismutase SOD activity in blood and liver of control (2.2 and 2.5) U/mg respectively while in its activity in treated blood and liver were (4 , 5.7 , 10.6) U/mg and (8.4 , 16.8 , 19.4) U/mg in three dose (3, 6, 9) µg/g respectively whereas catalase activity CAT in blood and liver of control (7.56 , 2664) U/mg respectively while in its activity in treated blood and liver were (28.08, 88.56, 30.48) U/mg and (57.36, 53.04, 47.28) U/mg in three dose (3, 6, 9) µg/g While glutathione peroxidase GPx in blood and liver of control (13.27 and 22.72) mol/ml respectively while in its activity in treated blood and liver were (32.5, 38.86, 31.34) mol/ml and (61.12, 64.53, 88.33) mol/ml in three dose (3, 6, 9) µg/g respectively Whereas the concentration of glutathione in blood and liver of control (30 , 46.33) µmol/ml respectively while in its activity in treated blood and liver were (51.53 , 53.4 , 38.4) µmol/ml and (53.33 , 43, 36.57) µmol/ml in three dose (3, 6, 9) µg/g and malondialdehyd concentration MDA in blood and liver of control (0.78 , 0,903) mol/ml respectively while in its activity in treated blood and liver were (2.76 , 4.61 , 6.66) mol/ml and (2.4 , 8.78 , 10.3) mol/ml in three dose (3, 6, 9) µg/g

Keywords: Toxins, Microcystin, *Anabaena circinalis*, Antioxidants, Lipid Peroxidation.

INTRODUCTION

Cyanobacteria are photoautotrophic prokaryotes microorganisms, capable of produce secondary metabolites named cyanotoxins, cyanobacterial toxins are released in the adjacent medium during bloom cell senescence and lysis, as well as via continual excretion. (Van der Merwe *et al.,* 2012).

It is possible to see blooms in various eutrophic to hypertrophic lakes, ponds, and rivers all over the world. Blooms are caused when there is an excessive amount of cyanobacterial development, which is then followed by the production of toxin. Cyanobacterial blooms are not only found in eutrophic reservoirs; the development of blooms and the release of toxins by toxin-producing cyanobacteria in water reservoirs can have a negative impact on the water quality. It has been shown that many different species and strains of bloom-forming cyanobacteria, which can be found in marine and freshwater environments, contain potentially hazardous or offensive compounds. These chemicals may be toxic to aquatic and terrestrial creatures, including people (Zegura and colleagues 2011 and Al-Shammari and colleagues 2020). Toxins can enter into body of organism by four main route including skin absorption, ingestion and injection, when toxic molecule enter the body of organism cause biological changes in biochemical characteristic of organism lead to alteration or damage in structure and function of macromolecules such as protein, lipid, antioxidants defense systems, the toxicity of cyanotoxins such as microcystins based on its chemical structure, ability of toxic substance to absorb on body of organisms, rate of entry and routs of exposure, sex and age of organism, organism health state, genetic of organism as well as environmental factors like temperature and pH value (Paerl and Otten 2013).

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Cyanotoxins are chemical compounds produced at stationary phase as secondary metabolites by cyanobacteria that exhibit toxicological and chemical properties and are capable of causing direct intoxication to aquatic and terrestrial organisms due to their ability to accumulate in organism tissue and transfer through the food chain, the optimum temperature for microcystins production was ranged between 20 – 32 C, 1800-3600 Lux of light intensity, while optimum PH was ranged between 6-9 and Cyanotoxins, especially microcystins, can be produced by a decrease in phosphorus, ferric Fe+2, and zinc Zn+2 nitrate (Teneva *et al.,* 2012 and Al-Shammari *et al.,* 2020).

MATERIALS AND METHODS

Culturing of *anabaena circinalis*

Anabaena circinalis was culturing according to method (Desikachary,1959, Tredici,2004)

Extraction and Purification of microcystin-LR

The *anabaena circinalis* was extracted according to (Namikoshi *et al.,* 1995) and Toxin purification has been carried out in accordance with Namikoshi *et al.,* (1993) and then, using the method of analysis, purification, and collection of Microcystin (Purdie *et al.,* 2009).

Injection of Microcystin to Selected Animals

Fish obtained in weights ranging from 200 to 20 g, then place 10 fish in each aquarium filled with 70 liters of dechlorinted tape water and leave it for three days to acclimate in appreciation laboratory conditions at temperature (252), pH (7.50.1), and appreciate light with continuously aeration

condition using electrical air pumps apparatus and then intraperitoneal injection of fish for dilution (Garcia *et al.,* 2012). Blood was drawn from a heart puncture, and some of it was placed in a gel tube, which was then centrifuged at 6000 r.p.m. for 10 minutes, and the serum was stored at -80°C for biochemical testing.

Antioxidants Enzymes

Superoxide Dismutase (SOD)

Autooxidation of Pyrogallol was used for assessing the activities of superoxide dismutase (Marklund and Marklund, 1974).

Catalase (CAT)

The catalase assay was performed in accordance with Aebi's protocol (1974, 1984).

Glutathione peroxidase (GPx)

The activity of glutathione peroxidase was examined using the method Hafemann *et al*., (1974).

Glutathione

Glutathione activity has been determined utilizing the method (Moron *et al.,* 1979).

Lipid peroxidation

Thiobarbituric acid test for MDA concentration had been used to measure lipid peroxidation (Aust, 1985).

Statistical analysis

The results of the study's data were examined by employing version 20 of the statistical package for social science (SPSS) in order to compute the means, standard deviations, and ANOVA's method for identifying the differences that were statistically insignificant.

RESULTS

During the span of this study, Anabaena circinalis was collected from Iraqi universities' research facilities, where it was grown on BG11 medium, which was the best medium to acquire biomass.

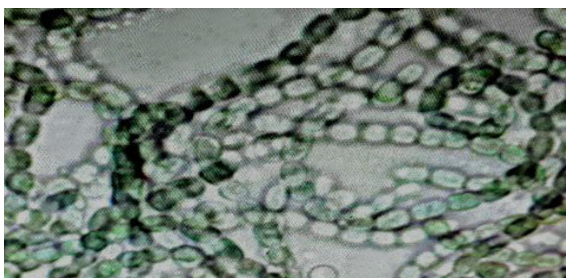


Figure 1. ***Anabaena circinalis***

Determination of growth curve of *Anabaena circinalis*

The growth density had been measured to identify the growth curve and determination stationary phase that produce microcystin-LR, figure (2) illustrates the growth curve of *A. circinalis,* Which are entered stationary phase in the eleven day.

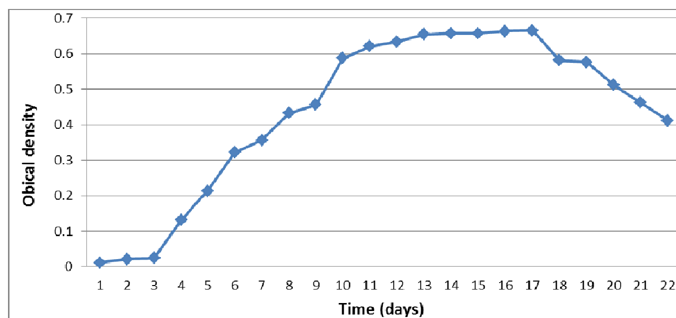
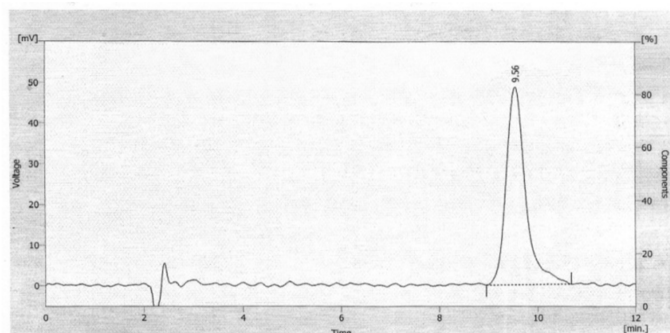


Figure 2 A. circinalis growth curve during the culture period

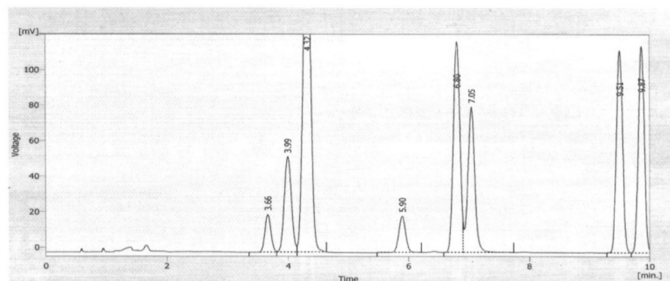
Preparative HPLC analysis and purification of the toxin Microcystin

Both the extraction of the crude A. circinalis and the purification of the microcystin were carried out. Comparing the peak area and retention time of an analytical standard of microcystin-LR with the peak area and retention time of extraction of each species of cyanobacteria using water: methanol: butanol and partially purified by silica gel column, followed by analysis by preparative HPLC to detect the presence of microcystin-LR, allowed for the determination of the toxin concentration. Figure 4 shows that the A. circinalis compound had a retention time of 9.51 minutes, and preparative HPLC was used to identify the compound once it had been thoroughly purified and collected.



	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]
1	9.557	1327.449	48.527	100.0	100.0	0.39
	Total	1327.449	48.527	100.0	100.0	

Figure 3. HPLC analysis standard of toxin Microcystin

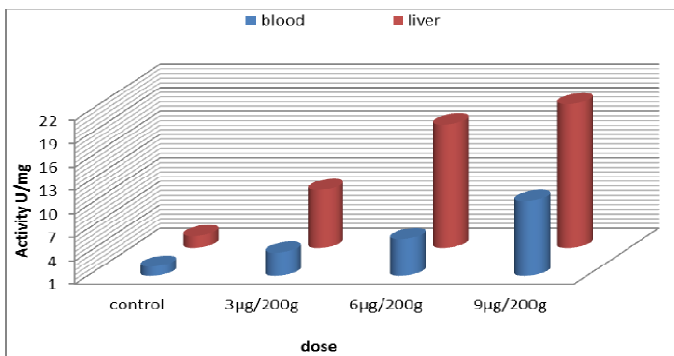


	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	3.660	146.419	21.218	2.6	3.0	0.11
2	3.990	440.865	53.571	7.9	7.6	0.13
3	4.320	1603.258	179.109	28.8	25.4	0.15
4	5.897	162.746	20.357	2.9	2.9	0.13
5	6.800	894.032	118.688	16.1	16.8	0.12
6	7.047	690.062	81.712	12.4	11.6	0.13
7	9.510	827.859	113.786	14.9	16.1	0.12
8	9.870	800.429	116.298	14.4	16.5	0.11
	Total	5565.672	704.739	100.0	100.0	

Figure 4. Preparative HPLC Analysis of *A. circinalis* microcystin toxin

The effect of Microcystin toxin on antioxidants levels in *C. carpio*

There are significant differences in SOD activity in blood and liver tissue between controls and treatments. SOD activity in blood was (2.2)U/mg and (2.5)U/mg in liver of control, while it was (4, 5.7, 10.6)U/mg in treated blood of three doses and (8.4, 16.8, 19.4) U/mg in treated liver of the same dose figure (5). Significant variations in CAT activity were seen between the control and treatment groups; CAT activity in the control group was (7.56) U/mg in blood and (26.64) U/mg in liver tissue. CAT activity in treated blood was (28.08, 88.56, 30.48)U/mg and activity in treated liver was (57.36, 53.04, 47.28)U/mg in the same dose correspondingly. (6). The activity of GPx in control blood was (13.27)mol/ml and in liver was (22.72)mol/ml, indicating significant variations between the two groups. While the activity of three doses was (32.5, 38.86, 31.34) mol/ml in treated blood and (61.12, 64.53, 88.33) mol/ml in treated liver tissue respectively figure (7). The GSH activity also appeared significant differences between control and treatment, activity of GSH in control was (30)µmol/ml in blood and (46.33) µmol/ml in liver, the GSH activity in treated blood was increased to (51.53, 58.4) µmol/ml in dose (3, 6) µg /200g b.w respectively and decreased to (38.4) µmol/ml in 9 µg /200g b.w , whereas activity in the treated liver was (52.33) µmol/ml in dose 3 µg /200g b.w and in doses (6, 9) µg /200g b.w was decreased to (43, 36.57) µg /200g b.w respectively figure (8). The concentration of malondialdehyd MDA appeared significant differences between control and treatments, The concentrations of malondialdehyd MDA in the control blood and liver were (0.78, 0.903) mol/ml, respectively, while the concentrations in the treated blood and liver were (2.76, 4.61, 6.66) mol/ml and (2.4, 8.78, 10.3) mol/ml in doses (3, 6, 9) g /200g b.w. figure (9).



Figur 5. The activity of superoxide dismutase in C. carpio blood and liver after continuous exposure to microcystin-

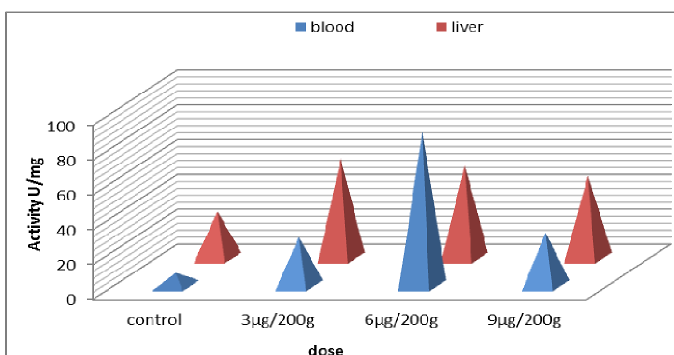


Figure 6. Catalase activity in C. carpio blood and liver afterwards a period of chronic exposure to microcystin

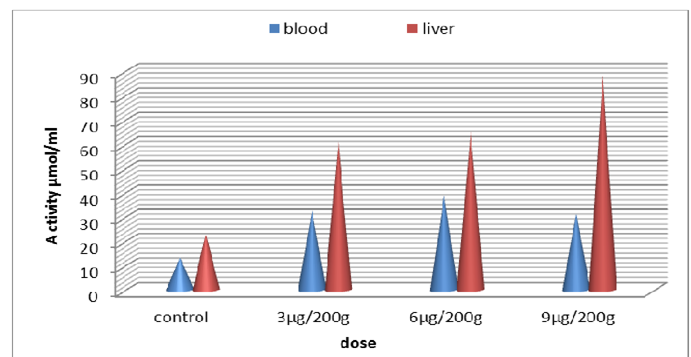


Figure 7. Glutathione peroxidase activity in C. carpio blood and liver after a period of continuous exposure to microcystin

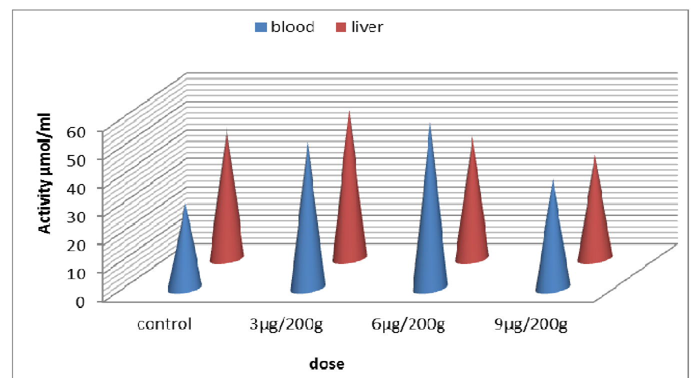


Figure 8. Activity of glutathione in blood and liver of *C. carpio* after chronic exposure period by Microcystin

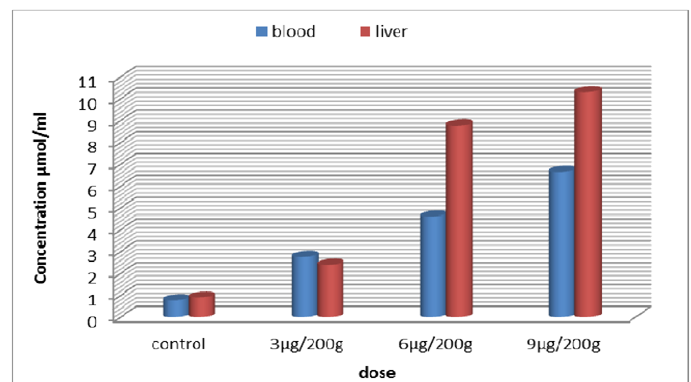


Figure 9. Concentration of malondialdehyde in blood and liver of *C. carpio* after chronic exposure period by purified microcystin

DISCUSSION

The current study's findings, the concentration of microcystin was identified in *A. circinalis* (74.832 µg/ml) so that the toxins of *A. circinalis* was highly purified and collected by preparative HPLC to observe it's effects on biochemical and molecular character in *C. carpio.* Zhang *et al.,* (1991). Lindholm and Meriluoto (2004) discovered the highest concentration of demethylmicrocystin-RR in O. agardhii and in microcystis bloom, the microcystin concentration was 2500 g/l in Ostera lake water in Aland. While Lalita et al. (2009) discovered the highest concentration of microcystin-RR in a strain of Microcystis, differences in microcystin concentration between different or the same species of cyanobacteria can be caused by intra- and interspecific variability, regulation of microcystin synthesis under different conditions, differences in toxin gene expression, growth phase, and environmental factors such as temperature, pH, and nutrition. (Martins and Vasconcelos 2011).

Biochemical markers in *C.carpio* fish as response to microcystin toxins

The study was revealed that microcystin induce oxidative stress in *C. carpio* fish that are intraperitoneally injected are made into the mid-ventral line just cranial to the vent during chronic period, the oxidative stress occurred as result of an imbalance between oxidants and antioxidants (Kamper *et al.,* 2009). the results of this study shown, liver was more affected than blood in *C. carpio* because the microcystin are potent hepatotoxins in mammals and fish (Jos *et al.,* 2005). The present study found that SOD activities in C. carpio blood and liver significantly elevated during the chronic period, but the highest activities of SOD in blood and liver because SOD plays a vital function in scavenging superoxide free radicals, which helps to maintain a balance between oxidants and antioxidants (Prieto *et al.,* 2006). The CAT activities significantly increased in blood and liver of *C.carpio* during chronic period, in *C. carpio*, CAT activities significantly elevated in blood and liver, This results coincide with study of Li *et al.,* (2003) showed stimulated SOD and CAT activities induced by microcystin-LR, the CAT and SOD activities increased 2.7 times compared with control during 96 hours. Pflugmacher et al. (2006) demonstrated that administering 5 g/l anatoxin-a boosted SOD activity and lipid peroxidation. While microcystin-LR boosted SOD activity in the blood and liver (Puerto *et al.,* 2009). By utilizing glutathione as a substrate, the GPx catalyzed the reduction of H2O2 to oxygen and water (Mates *et al.,* 1999).The current investigation found that GPx activity increased greatly in the blood and liver of C. carpio over time as a result of the response of antioxidant systems to microcystin and reflecting adaptation for oxidative conditions. (Li *et al.,* 2003).this results correspond with study reported increase the GPx activity in blood and liver of common carp after exposure to microcystin (Mohammed *et al.,* 2014). GSH is a key cofactor in many metabolic pathways and is required for the regulation of intracellular redox state. (Van Bladeren, 2000). During the chronic period, GSH activities in C.carpio blood were significantly increased because GSH levels increased as a result to overproduction to protect cells against oxidative stress, therefore the ability to enhance GSH synthesis in response to higher GSH usage demands whereas in liver, it's activities significantly decreased as compared to control groups due to GSH level decreased, which may be related to its involvements in detoxification of xenobiotic like microcystin (Van Bladeren, 2000). Pathogenesis disease in organisms is caused by oxidative stress, characterized by an imbalance in synthesis antioxidant defense and the formation of ROS, which leads for elevated in lipid peroxide levels. (Pasupathi *et al.,* 2009). The current investigation found that the content of malondialdihyde in blood and liver of *C. carpio* was significantly higher over the chronic period because microcystin-LR caused oxidative stress and elevated lipid peroxidation in primary hepatocytes (Ding *et al.,* 1998). Many studies have shown that lipid peroxidation and oxidative stress increase in organism tissues as a consequence of microcystin exposure (Prieto *et al.,* 2006), and the results of this study agreed with Jos *et al., (*2005) Microcystins were administered intraperitoneally to tilapia fish.

Conclusion

The generation of free radicals during exposure the fish to microcystin which cause oxidative stress on cells levels that lead characterized by an imbalance in synthesis antioxidant defense. The formation of reactive oxygen species so that avoiding the water that contain cyanobacteria for drinking and washing due to adversely affects of toxin may be kills animals including human.

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