Association Between Serum Microcystins Levels and Chronic Pelvic Inflammatory Disease: a Case-control Study

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Research Article

Keywords: microcystin-LR, CPID, inflammation

Posted Date: June 1st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-551070/v1

Abstract

Microcystins(MCs) have been reported to be closely related to the occurrence and development of inflammation by animal and cell experiments, but there are no study on the relationship between serum microcystin-LR(MC-LR) and chronic pelvic inflammatory disease (CPID) risk in populations. We designed a clinical case-control study to investigate the relationship between serum MC-LR and CPID risk. From October 2020 to March 2021, 50 patients diagnosed with CPID and 50 controls (frequency matched by age) were recruited from the First Hospital University of South China, in Hengyang, Central China. The basic information on lifestyle and history of disease was acquired through questionnaires. Blood samples were analyzed for MC-LR by ELISA. Binary logistic regression analyses and chi-square test were used to evaluate the effects of MC-LR on CPID risk. With the increase of serum MC-LR level (Q2, Q3 and Q4), the AOR of CPID risk increased (0.139, 0.167 and 0.040, respectively). The serum MC-LR(0.06 ~ 0.66μg/L) was an independent protective factor for CPID in humans, and the protective effect of concentrations ≥ 0.25μg/L was more obvious. Within the certain concentration range, MC-LR was an independent protective factor for the risk of CPID in humans, which will provide a scientific basis for the study of the relationship between serum microcystins and inflammation.

Introduction

At present, the global incidence of pelvic inflammatory disease(PID)is approximately 2%～12%[1], of which 10%～20% of PID patients may have secondary infertility, which seriously affects the reproductive health and quality of life of women of childbearing age[2–3]. In China, the most common gynecological diseases is genital tract infection (42.9%), and the proportion of chronic pelvic inflammatory disease(CPID) is the highest(4.1%)[4]. PID has become a major public health problem worldwide.

PID is an inflammation of the upper genital tract, that is caused by retrograde infection of the lower genital tract with a variety of microorganisms, including endometritis, salpingitis, parametritis, oophoritis, tuboovarian abscess and/or pelvic peritonitis[5–6]. CPID is a chronic inflammatory disease of the tissue in and around the female genitals, usually caused by incomplete treatment of acute pelvic inflammation or poor physical condition of the patient[7]. With the increasing incidence of PID, Chlamydia trachomatis and Neisseria gonorrhoeae are common pathogens[8], that have a high infection rate in sexually active women. Based on some data, the estimated prevalence of self-reported lifetime CPID was 4.4% in sexually experienced women of reproductive age (18–44 years)[9]. Female pelvic inflammatory disease is characterized by the diversity of pathogenic bacteria, high incidence rate, high recurrence rate, different severities, multiple complications, and high misdiagnosis rate, which places a severe financial burden on societies worldwide. Serious adverse health outcomes have been associated with CPID, including an increased risk of tubal factor infertility, ectopic pregnancy, and chronic pelvic pain. The cumulative risk of pelvic inflammation increases with age[10]. In Africa, the prevalence of female infertility caused by pelvic inflammatory disease is as high as 39.4%[11]. Therefore, PID is a key problem threatening female reproductive health.

Although previous studies have identified predisposing risk factors for CPID, such as multiple sexual partners, sexually transmitted infection, young age history of pelvic surgery and induced abortion, the influencing factors of up to 70% of cases are unknown[12]. Many animal and cell studies have indicated that microcystins(MCs)play a key role in the regulation of pro-inflammatory and anti-inflammatory cytokines in peripheral blood cells[13–14]. The inflammatory response caused by MCs varies with the concentration and time of its action, receiving much attentions recently. Related research reports that there are 53%, 28%, 48%, 41% and 54% of lakes in Europe, Africa, Central America, South America and Asia Pacific region, respectively, and 70% of lakes in China are eutrophic[15]. MCs are produced by cyanobacterial blooms and are hepatotoxic to animals and humans. In tropical and subtropical zones, MCs can be detected in aquatic products and water[16–18]. The level of serum microcystin in children and adults in China exceeded the standard, which was mainly related to dietary intake[13–14]. The etiology of PID is complex and varied, and microcystins may be one of the important reasons. At present, relevant studies in the population have not been reported.

Based on previous studies on the effect of MC-LR on inflammation in animals and cells, we hypothesized that a low concentration of MC-LR in human serum would promote the occurrence of inflammation, and that a certain concentration would inhibit the occurrence of inflammation. Therefore, we conducted a case-control study to assess the effect of MC-LR at different concentrations on CPID risk.

Materials And Methods

Study population. A case-control study was conducted to examine the relationship between exposure to microcystins and CPID risk in populations living in Hengyang and was approved by the Ethics Committee of the University of South China, Hengyang, Central China. We confirmed that all methods were performed in accordance with the relevant guidelines and regulations. Informed written consent was obtained from each participant. Case patients were diagnosed with CPID at the First Affiliated Hospital of the University of South China. The diagnostic criteria of female PID refer to the 2015 Centers for Disease Control and Prevention sexually transmitted disease (STD) treatment guidelines[1]. The inclusion criteria were a confirmed diagnosis of CPID(≥ 3 months) for patients who had lived in the Hengyang area for at least 5 years and were between the ages of 18 and 45. The exclusion criteria were subjects complicated with cardiovascular, liver, kidney, blood, diabetes, immune system or other serious primary diseases, or a history of using antibiotics, glucocorticoids and immunosuppressive drugs. Control subjects were nonpelvic inflammatory patients from the Department of Ophthalmology and Otorhinolaryngology in the same hospital who had no history of gynecological surgery, abdominal surgery, tumor, or severe cardiovascular disease and had no history of antibiotics, glucocorticoids, or immunosuppressants in the past year. Cases and controls were frequency-matched by age (± 5 years) and were recruited in the same period. Patients who failed to answer the questions in the questionnaire were excluded from recruitment of cases and controls. Fifty CPID case patients and 50 control subjects participated in this study, from October 2020 to March 2021.

Study questionnaire. The study questionnaire collected information on demographic characteristics and related risk factors for CPID (Table 1), including age, education level, marital status, income, reproductive history, related disease history, lifestyle, and sexually related information. All of the study participants were interviewed in person by trained investigators.

Collection of clinical blood samples. Peripheral blood samples from cases and controls were collected under vacuum (BD V acutainer® SSTTMII advance) in the morning, and the serum was separated following centrifugation at 3000×g for 15 min. All of the serum samples were transferred to 2 mL plain collection tubes, and kept frozen at -80°C.

MC-LR levels in serum. MC-LR levels in serum were determined with direct competitive ELISA kits [(#20–0068), Beacon Analytical Systems Inc., USA], by strictly following the kit instructions. (a) Fifty microliters of MCY -HRP enzyme conjugate solution was added to each well. (b) Fifty microliters of negative control or standard solution (0.1, 0.3, 0.8, 1.0 and 2.0 ng/mL). (c) Fifty microliters of each sample was added into the assigned well. (d)The plate was lightly swirled to mix the contents thoroughly and incubated at 37°C for 30 minutes in the dark. (e)The plate was washed 5 times with an automated microplate washer. (f) The plate was inverted and lightly patted on absorbent paper towels to remove the remaining solution in the wells, followed by the step of addition of 100 μL of substrate solution to each well and light shaking. (g)After incubation for 30 minutes at 37°C in the dark, 100 μL of stop solution was added to each well. (h) The plate was read on a microtiter reader at 450 nm. (i) A five-point calibration curve for standard MC-LR was plotted at concentrations of 0.1 ~ 2.0 ng/mL. Log-linear regression was used to plot the absorbance of MC-LR which corresponds to its concentration. (j) All samples were repeated in duplicate, and the average values calculated. The detection limit of MC-LR was 0.01 ng/mL. To confirm the reliability of the ELISA results, a recovery test with added microcystin standard material in samples was conducted. The average recovery from serum samples was 90%, with an RSD of 9.0%.

Statistical Analysis. The SPSS 26.0 was used for statistical analysis. Descriptive analysis was used to determine the mean, standard deviation, median, range and percentile. We performed descriptive statistics on the distributions of participants' serum MCs, and personal serum MCs were compared using the chi-square test. To evaluate the relationship between MC exposure and CPID risk, we divided participants into the " < 0.15 μg/L", "0.15–0.19 μg/L", "0.20–0.24 μg/L" and "≥0.25 μg/L" groups with different MC levels by quartiles. Binary logistic regression was used to reveal the possible association between MC-LR and CPID risk factors adjusting for the number of pregnancies and abortions, history of abdominal surgery and gynecological surgery, frequency of sex (times/week), and ≥ 2 lifetime sex partners, and the adjusted odds ratio (AOR) and 95% confidence interval (CI) values were calculated. All statistical tests were two-sided, and a P value < 0.05 was considered to be statistically significant.

Results

Baseline information. A total of 50 CPID cases and 50 controls were enrolled in the study with mean ages of 32.04 ± 5.98 years and 28.14 ± 4.58 years, respectively. The baseline demographic characteristics and

other factors for CPID cases and controls are summarized in Table 1. No significant differences were found between CPID cases and controls with regard to income, number of pregnancies, number of abortions, frequency of sex(times/week)and contraception use. However, significant differences were observed in age, educational level, marital status, history of abdominal surgery, history of gynecological surgery, ≥ 2 lifetime sex partners, sex during menstruation and MC level. In addition, the median MC level (μg/L) in CPID controls (0.247 μg/L) was significantly ($P < 0.001$) higher than that in cases (0.196 μg/L).

Table 1
Demographic characteristics of CPID case(N = 50) and controls (N = 50).

Valiable [n (%)]	CPID cases	controls	*P*-value
Age (year)			<0.001[a]
< 25	5(10.0)	12(24.0)	
25~	28(56.0)	35(70.0)	
≥ 35	17(34.0)	3(6.0)	
Educational level			0.037[a]
Senior and below	23(46.0)	13(26.0)	
Junior college and above	27(54.0)	37(74.0)	
Income(¥/person/year)			0.085[a]
< 3000	18(36.0)	29(58.0)	
3000~	17(34.0)	12(24.0)	
≥ 5000	15(30.0)	9(18.0)	
Marital status			0.033[b]
unmarried	2(4.0)	8(16.0)	
married	48(96)	42(84.0)	
Number of pregnancies			0.575[b]
0	11(22.0)	14(28.0)	
1~	22(44.0)	26(52.0)	
≥ 3	17(34.0)	10(20.0)	
Number of abortions			0.083[b]
0	26(52.0)	38(76.0)	
1~	18(36.0)	10(20.0)	
≥ 3	6(12.0)	2(4.0)	
History of abdominal surgery			0.025[a]
No	41(82.0)	48(96.0)	
Yes	9(18.0)	2(4.0)	

a From c^2 test; b From fisher's exact test.

Valiable [n (%)]	CPID cases	controls	*P*-value
1 or more	14(28.0)	33(66.0)	
History of gynecological surgery			<0.001[a]
No	17(34.0)	36(72.0)	
Yes	33(66.0)	14(28.0)	
Frequency of sex(times/week)			0.515[b]
0	4(8.0)	5(10.0)	
1~	33(66.0)	35(70.0)	
≥ 3	13(26.0)	10(20.0)	
Contraception use			0.836[a]
None	31(62.0)	32(64.0)	
1 or more	19(38.0)	18(36.0)	
≥ 2 lifetime sex partners			<0.001[b]
No	5(10.0)	14(28.0)	
Yes	45(90.0)	36(72.0)	
Sex during menstruation			<0.001[b]
No	4(8.0)	3(6.0)	
Yes	46(92.0)	47(94.0)	
MC level (μg/L)			<0.001[a]
< 0.15	16(32.0)	5(10.0)	
0.15~	16(32.0)	10(20.0)	
0.20~	11(22.0)	11(22.0)	
≥ 0.25	7(14.0)	24(48.0)	
a From c^2 test; b From fisher's exact test.			

Association between microcystins and CPID. The concentration range of serum microcystins was 0.06 ~ 0.66 μg/L, and the median was 0.21 μg/L in the study population. Table 2 shows the associations between serum MC-LR and CPID risk. CPID controls had a higher level of serum MC-LR than cases by the

chi-square test (P < 0.001). The median serum MC level was 0.196 μg/L for CPID cases, and 0.247 μg/L for controls. A clear relationship between an increased serum MC-LR level and CPID risk was observed. Compared with the lowest MC-LR group (Q1), the AORs of CPID risk were 0.139(95% CI, 0.025–0.790), 0.167(95% CI, 0.032–0.865), and 0.040(95% CI, 0.007–0.243) for groups Q2, Q3, and Q4, respectively. The results suggested that serum MC-LR was an independent protective factor for CPID development in our study. In addition, the prevalence of pelvic inflammation decreased with an increase of the concentration of serum microcystins (0.06 ~ 0.66μg/L), based on the chi-square test trend with statistical significance ($P < 0.001$)

Table 2
Associopmentation analyses between MC-LR and risk of CPID development:OR(95% CI) using binary logistic regression

Serum MC-LR	CPID cases	control	Crude OR(95% CI)	P-value	Adjusted OR(95% CI)	*P*-valuea
Quartile[n(%)]						
Q1(< 0.15μg/L)	16(32.0)	5(10.0)	1.000(reference)		1.000(reference)	
Q2(0.15 ~ 0.19μg/L)	16(32.0)	10(20.0)	0.500(0.139–1.794)	0.288	0.139(0.025–0.790)	0.026
Q3(0.20 ~ 0.24μg/L)	11(22.0)	11(22.0)	0.313(0.085–1.154)	0.081	0.167(0.032–0.865)	0.033
Q4(≥ 0.25μg/L)	7(14.0)	24(48.0)	0.091(0.025–0.338)	0.001	0.040(0.007–0.243)	0.001
a Adjustment for age, educational level, marital status, history of abdominal surgery, history of gynecological surgery, ≥ 2 lifetime sex partners, having sex during menstruation.						

Discussion

In our study, we found that the study populations were exposed to microcystins. The concentration range of serum microcystins was 0.06 ~ 0.66 μg/L, and the median was 0.21 μg/L. Our study site is located in Central China, with a subtropical monsoon humid climate, which is suitable for the growth and propagation of microcystins. Microcystins pollution is not a rare phenomenon in China.

In some areas of China, microcystin pollution is a serious phenomenon, especially in tropical and humid areas, among which Chao Lake is the region that is most seriously polluted by microcystins, where the serum level of MC-LR in fishermen can be up to 0.38 μg/L[19]. Many researchers have pointed out that MCs may affect human health in the following ways: contaminated water and aquatic products, dietary supplements, body contact, hemodialysis and inhalation[20–21]. Therefore, the reasons for the study populations exposure to microcystins may be as follows. First, water and aquatic products(such as fish, snails, soft-shelled turtles, and ricefield eel.) are contaminated by MCs. Second, residents lack knowledge of cyanobacteria and microcystins, so they ignore daily contact in everyday life. Finally, residents'

exposure to microcystins is also affected by their behavior and lifestyles, such as their preference for aquatic products and well water.

We conducted a case-control study in women of childbearing age and found that the average level of serum MC-LR in CPID control subjects was higher than that in case patients. Microcystins(0.06 ~ 0.66μg/L) were a protective factor for CPID risk. Previous animal experiments and clinical observations have reported that MCs can promote and inhibit inflammation, but CPID epidemiological studies are rare. The potential mechanism for the association between MCs and CPID is complicated. First, microcystins caused imbalance of pro-inflammatory and anti-inflammatory cytokines in vitro. According to previous studies, IL-1, TNF-α, IFN-g and GM-CSF are recognized as the main inflammatory cytokines and participate in resistance to xenoantigens, and their mRNA levels were inhibited by MC-LR in a dose dependent manner[22]. Several studies have shown that the expression of pro-inflammatory cytokines were suppressed and anti-inflammatory cytokines were promoted in blood with microcystins at higher concentrations[16–17, 23].The downregulation of inflammatory levels caused by MC-LR may be related to escaping immune monitoring and these changes may weaken the function of macrophages.

In contrast, the lower concentration of MCs promoted pro-inflammatory and suppressed anti-inflammatory in blood, and the imbalance of pro-inflammatory cytokines expression were more rapid than that of anti-inflammatory cytokines. This change might be related to the low concentration of microcystins inducing macrophages to activate the NF-κB and ERK1/2 pathways in autophagy[24–25]. This serve as a possible explanation for the low incidence rate of CPID with MCs at the higher concentration (≥ 0.25 μg/L) in this study, but it is notesd that, the low concentration levels in animal and cell experiments are much higher than those in human serum; for example, the incubation of leukocytes isolated from blood and head kidney with a high concentration of MC-LR (0.1 mg/ml) caused a significantly increased ($P < 0.05$) expression of IL-10 and TNF-α, while a low concentration (0.01 mg/ml) did not induce the expression of IL-10 and TNF-α[23]. In addition, the inhibition of MC-LR on inflammation is closely related to the time of action. For example, medium and high concentration of MC-LR (40 ~ 80 g/L) could promote the expression of the pro-inflammatory cytokines- TNF-α, IL-1β and IFN-γ after 48 hours, and inhibit the expression of the anti-inflammatory cytokines- IL-4, IL-10 and IL-13 after 72 hours[26]. The anti-inflammatory effect of microcystin is significantly different based on its concentration and time. Second, the ratios of Th, Tc and double-positive T cells were changed at certain concentrations of microcystins, which affected the adaptive immune response of the body. NK cells and gamma-delta T cells were increased at higher concentrations of microcystins, which affected the innate immune responses. NK cells and γδ T cells are the bridge between adaptive and innate immune response in vivo. Therefore, human body exposure to microcystins generates an adaptive immune response, stimulating the production of antibodies and inhibiting inflammation[27]. Third, MCs can easily cross the intestine-blood barrier and enter various cells through OATP transporters[28]. Some studies have shown that subchronic exposure to MC-LR increased the gut microbial diversity in the caecum, including Porphyromonadacese, Lachnospiraceae, Ruminococcaceae and Prevotellaceae, in both Bacteriodetes and Firmicutes. Firmicutes were absolutely predominant in caecum and colon[29]. Lactobacillus is a species of Firmicutes, and the increase in intestinal Lactobacillus is beneficial for maintaining the

microecological balance, and preventing retrograde infection of intestinal flora, thus promoting the health of the body or treatment of certain diseases. Therefore, microcystins have a certain effect on changes in the microclimate in the genital tract. Finally, phycocyanin is produced by cyanobacteria, which are rare natural nutrients. Previous studies have proven that phycocyanin has anti-inflammatory and anti-tumor effects and increases the richness and diversity of the microbiota[30–32]. However, when phycocyanins is extracted, a large amount of microcystins may be released. Phycocyanin can enter the human body along with microcystins through the consumption of cyanobacteria-contaminated water and food and plays an anti-inflammatory role and modulates genital tract microbiota. Further research is required to determine the concentration of phycocyanin in the human body, and its role, and underlying mechanisms of phycocyanin in regulating genital tract microbiota.

Our study has several limitations. First, the sample size is small. Second, we did not measure all types of microcystins in serum. Third, we did not perform additional experiments to explore the possible mechanism of association between MCs and CPID. We will conduct related studies in the future.

Conclusions

In conclusion, the concentration of microcystins in serum is a protective factor against pelvic inflammation within a certain range(0.06 ~ 0.66 μg/L) in our study population, especially when the serum microcystins were more than 0.25 μg/L, the protective effect was more obvious. Future research should focus on the relationship between microcystin exposure and the risk of pelvic inflammatory disease in the population and its mechanism.

Declarations

Acknowledgements:

We thank American Journal Experts(https://www.aje.con) for editing this manuscript.

Author contributions statement:

All authors conceived and designed the study. N.Z and H.G. conducted the surgeries. S.S.X., Y.H.P., X.L.W. and J.T. conceived the questionnaire and experiment(s). All authors analyzed the data and wrote the paper. All authors contributed to manuscript revision. All authors reviewed the manuscript and agreed to be held accountable for the content therein.

Conflict of Interest:

The authors declare no conflict of interest.

Data availability:

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Workowski, K.A. & Bolan, G.A. Sexually transmitted diseases treatment guidelines. MMWR Recomm Rep. 64, 1-137 (2015) .
2. Goller, J.L. *et al*. Rates of pelvic inflammatory disease and ectopic pregnancy in Australia, 2009-2014: ecological analysis of hospital data. Sex Transm Infect. 94, 534-541 (2018).
3. Ness, R.B. *et al*. Prediction of pelvic inflammatory disease among young, single, sexually active women. Sex Transm Dis. 33, 137-42 (2006).
4. Liu, B. *et al*. Clinical effect of integrated traditional Chinese and western medicine in the treatment of 46 patients with acute biliary pancreatitis. Hebei Traditional Chinese medicine. 35, 1580-1582 (2013).
5. Ford, G.W. & Decker, C.F. Pelvic inflammatory disease. Dis Mon. 62, 301-5 (2016).
6. Ross, J., Guaschino, S., Cusini, M. & Jensen, J. 2017 European guideline for the management of pelvic inflammatory disease. Int J STD AIDS. 29, 108-114 (2018).
7. Wang, C., Chen, J., Xiao, Y. & Shen, Q. Guizhi Fuling wan for chronic pelvic inflammatory disease protocol: A protocol for systematic review and meta analysis. Medicine (Baltimore). 99, e23549 (2020).
8. Hoof, K. Entzündliche Erkrankungen des weiblichen Genitale [Pelvic inflammatory disease]. Ther Umsch. 64, 365-368 (2007).
9. Kreisel, K., Torrone, E., Bernstein, K., Hong, J. & Gorwitz, R. Prevalence of Pelvic Inflammatory Disease in Sexually Experienced Women of Reproductive Age - United States, 2013-2014. MMWR. 66, 80-83 (2017).
10. Simms, I., Rogers, P.A. & Nicoll, A. The influence of demographic change and cumulative risk of pelvic inflammatory disease on the incidence of ectopic pregnancy. Epidemiol Infect. 119, 49-52 (1997).
11. Abebe, M.S., Afework, M. & Abaynew, Y. Primary and secondary infertility in Africa: systematic review with meta-analysis. Fertil Res Pract. 6, 20 (2020).
12. Haggerty, C.L. *et al*. Identification of novel microbes associated with pelvic inflammatory disease and infertility. Sex Transm Infect. 92, 441-446 (2016).
13. Zurawell, R.W., Chen, H., Burke, J.M. & Prepas, E.E. Hepatotoxic cyanobacteria: a review of the biological importance of microcystins in freshwater environments. J Toxicol Environ Health B Crit Rev. 8, 1-37 (2005).
14. Liu, W. *et al*. Environmental Microcystin Exposure Increases Liver Injury Risk Induced by Hepatitis B Virus Combined with Aflatoxin: A Cross-Sectional Study in Southwest China. Environ Sci Technol. 51, 6367-6378 (2017).
15. Alastair, D.J. & Toxic Cyanobacteria in Water, edited by Ingrid Chorus and Jamie Bartram. J Appl Phycol. 11, 595 (1999).

16. Peng, L. *et al*. Health risks associated with consumption of microcystin-contaminated fish and shellfish in three Chinese lakes: significance for freshwater aquacultures. Ecotoxicol. Environ. Saf. 73, 1804–11 (2010).
17. Zhang, D. *et al*. Occurrence and spatial distributions of microcystins in Poyang Lake, the largest freshwater lake in China. Ecotoxicology. 24, 19–28 (2015).
18. Wu, S. *et al*. Field studies on the environmental factors in controlling microcystin production in the subtropical shallow lakes of the Yangtze River. Bull. Environ. Contam. Toxicol. 80, 329–34 (2008).
19. Peng, L. *et al*. Health risks associated with consumption of microcystin-contaminated fish and shellfish in three Chinese lakes: significance for freshwater aquacultures. Ecotoxicol Environ Saf. 73, 1804-1811 (2010).
20. Alosman, M., Cao, L., Massey, I.Y. & Yang, F. The lethal effects and determinants of microcystin-LR on heart: Aminireview. 8, 1-10 (2020).
21. Massey, I.Y. *et al*. Exposure routes and health effects of microcystins on animals and humans: A mini-review. Toxicon. 151, 156–162 (2018).
22. Chen, T. *et al*. Analysis of immunomodulating nitric oxide, iNOS and cytokines mRNA in mouse macrophages induced by microcystin-LR. Toxicology. 197, 67-77 (2004).
23. Rymuszka, A. & Adaszek, Ł. Pro- and anti-inflammatory cytokine expression in carp blood and head kidney leukocytes exposed to cyanotoxin stress--an in vitro study. Fish Shellfish Immunol. 33, 382-388 (2012).
24. Adamovsky, O. *et al*. Immunomodulatory Potency of Microcystin, an Important Water-Polluting Cyanobacterial Toxin. Environ Sci Technol. 49, 12457-12464 (2015).
25. Adegoke, E.O. *et al*. Selenium (Na(2)SeO(3)) Upregulates Expression of Immune Genes and Blood-Testis Barrier Constituent Proteins of Bovine Sertoli Cell In Vitro. Biol Trace Elem Res. 185, 332-343 (2018).
26. Adegoke, E.O. *et al*. Microcystin-leucine arginine (MC-LR) induced inflammatory response in bovine sertoli cell via TLR4/NF-kB signaling pathway. Environ Toxicol Pharmacol. 63, 115-126 (2018).
27. Palikova, M. *et al*. In vivo effects of microcystins and complex cyanobacterial biomass on rats (Rattus norvegicus var. alba): changes in immunological and haematological parameters. Toxicon. 73, 1-8 (2013).
28. Zeller, P., Quenault, H., Huguet, A., Blanchard, Y. & Fessard, V. Transcriptomic comparison of cyanotoxin variants in a human intestinal model revealed major differences in oxidative stress response: effects of MC-RR and MC-LR on Caco-2 cells. Ecotoxicol Environ Saf. 82, 13-21 (2012).
29. Chen, J. *et al*. Effects of microcystin-LR on gut microflora in different gut regions of mice. J Toxicol Sci. 40, 485-94 (2015).
30. Shih, C.M., Cheng, S.N., Wong, C.S., Kuo, Y.L. & Chou, T.C. Antiinflammatory and antihyperalgesic activity of C-phycocyanin. Anesth Analg. 108, 1303-10 (2009).

31. Madhyastha, H.K., Radha, K.S., Nakajima, Y., Omura, S. & Maruyama, M. uPA dependent and independent mechanisms of wound healing by C-phycocyanin. J Cell Mol Med. 12, 2691-703 (2008).
32. Bannu, S.M. *et al*. Potential Therapeutic Applications of C-Phycocyanin. Curr Drug Metab. 20, 967-976 (2019).