IOCT 2025 Conference

The 3rd International Online Conference on Toxins

10–12 September 2025 | Online

MDPI

Identification of potential metabolites of Cylindrospermopsin and Microcystin-LR and effects on brain oxidative stress biomarkers in rats after oral exposure to their mixture

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INTRODUCTION & AIM

Cylindrospermopsin (CYN) and microcystin-LR (MC-LR) are cyanotoxins of significant concern due to their widespread occurrence and toxic potential. Among exposure routes, ingestion through contaminated water and food is particularly relevant. Although CYN and MC-LR are primarily classified as cytotoxin and hepatotoxin, respectively, both cyanotoxins have shown potential neurotoxic effects. In addition, a recent study has reported CYN metabolites in the brain of orally exposed rats. However, to our knowledge, the combined impact of CYN and MC-LR on the brain remains unexplored.

This study aimed to identify CYN, MC-LR, and their potential metabolites in the brain of Wistar rats after oral exposure to dose combinations of pure CYN and MC-LR (7.5+75, 23.7+237, and 75+750 µg/kg b.w.) by Ultra-High Performance Liquid Chromatography coupled to a Tandem Mass Spectrometry System (UHPLC-MS/MS). Furthermore, several oxidative stress biomarkers were assessed.

METHODS

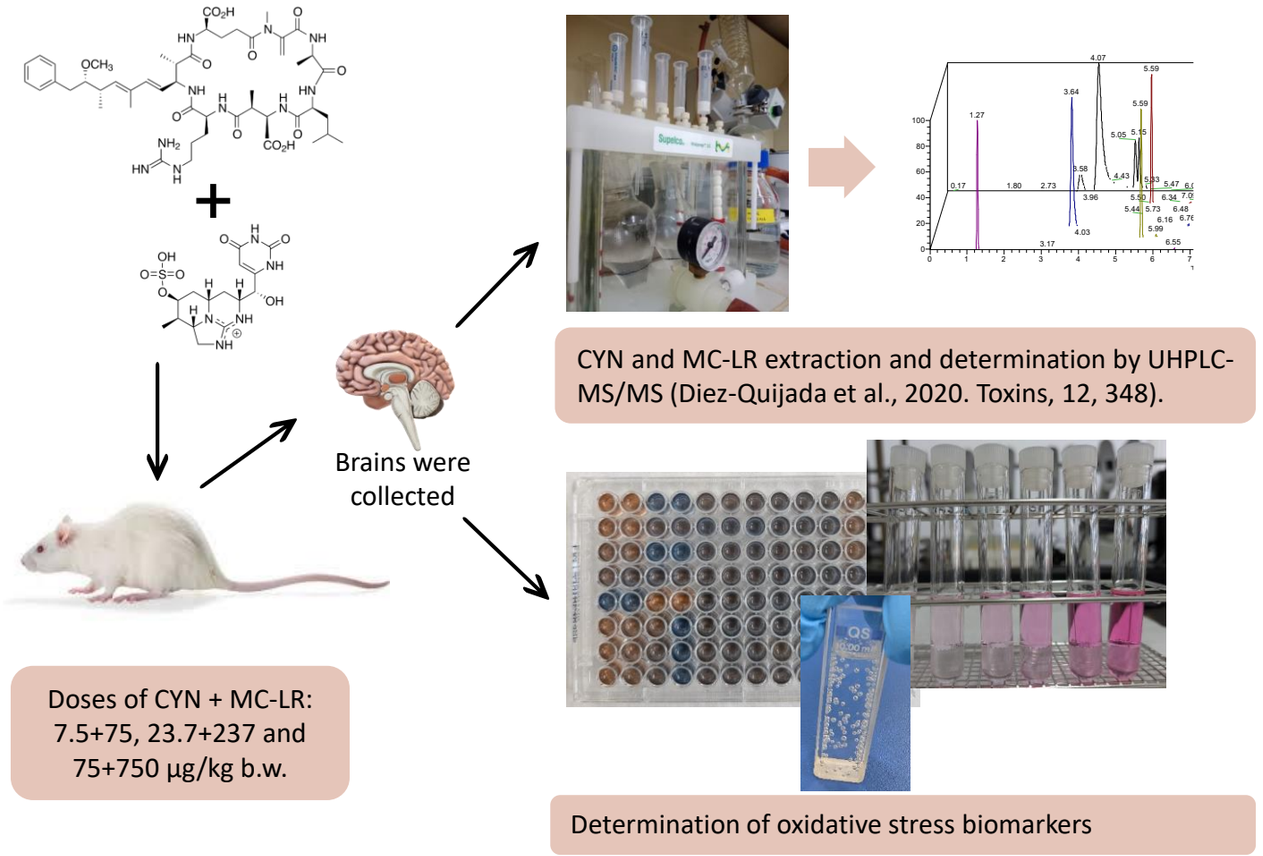

Figure 1. Experimental design and treatment of Wistar rats with CYN and MC-LR. Five animals per group and sex were used (n=5).

RESULTS

Although the parental compounds (CYN and MC-LR) were not detected in the brain, several potential metabolites produced by different metabolic pathways were identified. Some of these are shown in tables 1 and 2.

Table 1: Potential CYN-derived compounds in brain samples from rats orally exposed to different doses of CYN+MC-LR mixtures (7.5+75, 23.7+237, and 75+750 µg CYN+MC-LR /kg b.w.). Det.: detected. n.d.: not detected

CYN-derivated compound	Biotransformations	Composition Change	m/z	RT (min)	7.5 µg CYN/kg b.w.		23.7 µg CYN/kg b.w.		75 µg CYN/kg b.w.	
					♀	♂	♀	♂	♀	♂
$C_{15}H_{19}N_5O$	Dehydration, Nitro Reduction	- (H_2O_6S)	286.165	3.777	n.d	Det.	Det.	n.d	n.d	Det.
$C_{18}H_{24}N_6O_7S_2$	Dehydration, Dehydration, Cysteine Conjugation 2	+ (C_3H_3NS)	523.104	0.555	Det.	n.d	n.d	n.d	Det.	n.d
$C_{31}H_{51}N_5O_6$	Hydration, Palmitoyl Conjugation	- (OS) + ($C_{16}H_{30}$)	590.392	6.062	n.d	Det.	Det.	n.d	n.d	n.d

Table 2: Potential MC-LR-derived compounds in brain samples from rats orally exposed to different doses of CYN+MC-LR mixtures. (7.5+75, 23.7+237, and 75+750 µg CYN+MC-LR /kg b.w.). Det.: detected. n.d.: not detected

MC-LR-derivated compound	Biotransformations	Composition Change	m/z	RT (min)	75 µg MC-LR/kg b.w.		237 µg MC-LR/kg b.w.		750 µg MC-LR/kg b.w.	
					♀	♂	♀	♂	♀	♂
$C_{50}H_{80}N_{10}O_{14}$	Hydration, Methylation	+ (CH_6O_2)	523.298	8.346	Det.	n.d	Det.	n.d	Det.	Det.
$C_{49}H_{80}N_{10}O_8$	Nitro Reduction, Nitro Reduction, Reduction	-(O_4) + (H_6)	959.608	8.454	Det.	n.d	Det.	n.d	Det.	n.d
$C_{50}H_{80}N_{10}O_8$	Nitro Reduction, Nitro Reduction, Methylation	-(O_4) + (CH_6)	949.624	8.338	Det.	n.d	Det.	n.d	Det.	n.d
$C_{49}H_{80}N_{10}O_8$	Hydration, Oxidation	+ (H_2O_2)	515.283	8.360	Det.	n.d	Det.	n.d	Det.	n.d

Moreover, sex-dependent differences were observed in oxidative stress biomarkers. In males, exposure to the intermediate dose (23.7 + 237 µg/kg) significantly increased lipid peroxidation (LPO) levels (1.3-fold), as well as superoxide dismutase (SOD) (1.3-fold) and catalase (CAT) (1.6-fold) activities compared to controls. In females, only changes in LPO levels were observed, whit a significant increase at all doses tested.

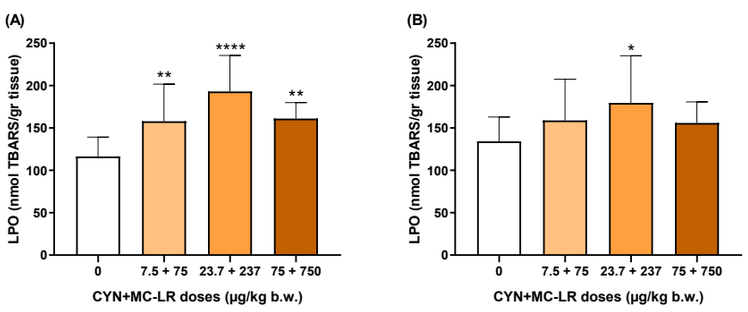

Figure 2. LPO levels in brain of female (A) and male (B) Wistar rats exposed to CYN+MC-LR doses of 7.5+75, 23.7+237 and 75 +750 µg/kg b.w. *p<0.05, **p<0.01 and ***p<0.0001 compared to the control group.

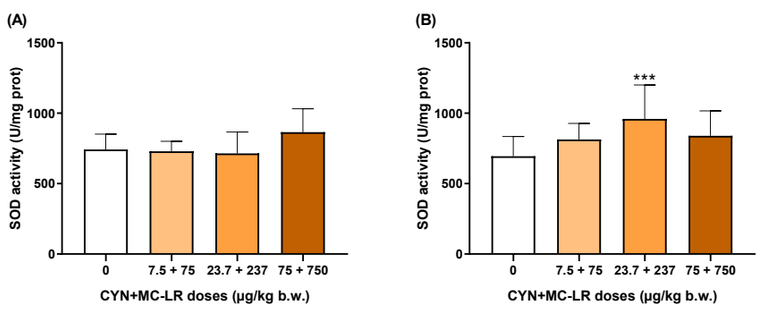

Figure 3. SOD activity in brain of female (A) and male (B) Wistar rats exposed to CYN+MC-LR doses of 7.5+75, 23.7+237 and 75 +750 µg/kg b.w. ***p<0.001 compared to the control group.

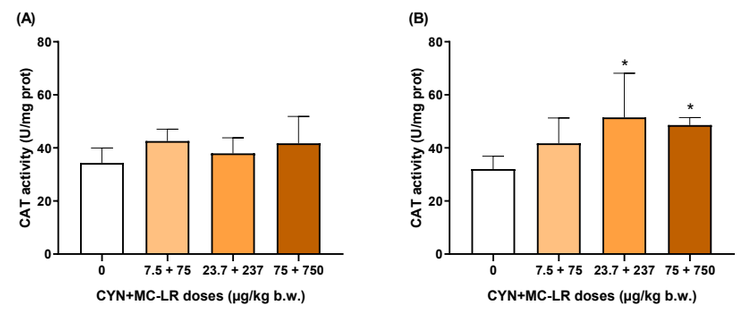

Figure 4. CAT activity in brain of female (A) and male (B) Wistar rats exposed to CYN+MC-LR doses of 7.5+75, 23.7+237 and 75 +750 µg/kg b.w. *p<0.05 compared to the control group.

CONCLUSION

These findings suggest that oral exposure to CYN+MC-LR mixtures may cause neurotoxic effects in rats and highlight the importance of considering sex as a biological variable in cyanotoxins toxicological assessment.

ACKNOWLEDGEMENT

The authors would like to thank the Project PID2023-147444OB-I00 funded by MICIU/AEI/10.13039/501100011033 and FEDER, UE. Plata-Calzado thanks the Consejería de Transformación Económica, Industria, Conocimiento y Universidades de la Junta de Andalucía for awarding the predoctoral contract (PREDOC_00447) in the Predoctores 2021 grant.