ABSTRACT

Toxic cyanobacterial blooms have become a growing threat to fresh and brackish waters in the recent years with their ever-increasing frequency and intensity. These blooms not only affect the regional marine flora and fauna but also the socio-economic health of the surrounding region. Cyanobacteria or blue green algae are unicellular, autotrophic prokaryotes which can cause dense, noxious blooms. Some of these cyanobacteria produce cyanotoxins as secondary metabolites. One of the most common and potent toxins produced by these blooms is Microcystin-LR (MC-LR) which is a known hepatotoxin. Although the current World Health Organization (WHO) guidelines for safe exposure to MC-LR have been extrapolated based on studies done in healthy animals, not much has been studied about the effects of these toxins in the setting of pre-existing conditions like Non-alcoholic Fatty Liver Disease (NAFLD).

We have demonstrated that chronic or short-term exposure to low doses of MC-LR induced hepatotoxicity marked with significant hepatic micro-vesicular lipid accumulation and oxidative stress in mice with genetic or diet-induced NAFLD. Additionally, NAFLD mice exposed to MC-LR demonstrated impaired hepatic metabolism and excretion of MC-LR compared to MC-LR exposed healthy mice. One of the main observations of MC-LR exposure in these animals was a significant increase in oxidative stress. Therefore, we next treated the mice with antioxidants like N-acetyl cysteine (NAC), a clinically used drug and pNaKtide, an in-house peptide, both of which are known to augment the glutathione pathway and showed that antioxidant therapy significantly reduced the hepatoxicity induced by MC-LR in diet-induced NAFLD mice. These observations were parallel to those observed in *in vitro* experiments with both murine and human hepatocyte cell lines indicating the role played by hepatocytes in toxin uptake and metabolism. These results manifest that treatment with antioxidants could possibly be a therapeutic approach to alleviate the damage done by cyanotoxins like MC-LR. Our studies have also shown that animals that were exposed to the toxin even for a short duration could develop an antibody-mediated immune response which for the first time can be used clinically as a diagnostic tool to monitor exposure to toxic cyanobacterial blooms in humans as well as animals.

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DISSERTATION PRESENTATION

by

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September 23rd, 2021

Mechanism of Hepatic Injury Susceptibility and Immune Response to Cyanotoxin Exposure

Ph.D. in Biomedical Sciences

AWARDS/ LEADERSHIP

2021 Invited talk at International Association for Great Lakes Research

2021 Recipient of two Trainee Travel Awards to attend Central Society for Clinical and Translational Research

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2019 Advanced Leadership Academy Certificate Program, College of Business and Innovation

2019 Recipient of Trainee Travel Award to attend Central Society for Clinical and Translational Research

2018 Finalist in the Three Minute Thesis® (3MT®) 2018 competition (inaugural year) at the University of Toledo

2018 Recipient of American Federation of Medical Research Scholar Award at Central Society for Clinical and Translational Research Conference

2017 Graduate Research Mentor for National Science Foundation Research Experience for Undergraduates, Health Effects of Harmful Algal Bloom toxins in Vulnerable Populations with Pre-existing Diseases

PUBLICATIONS & PATENT

Kucheriavaia D, Veličković D, Peraino NJ, **Lad A**, Kennedy DJ, Haller ST, Westrick J, Isailovic D. Toward Revealing Microcystin Distribution in Mouse Liver Tissue using MALDI-MS Imaging. *Toxins*. 2020 (In Revision)

Su RC, Meyers CM, Warner EA, Garcia JA, Refsnider JM, **Lad A**, Breidenbach JD, Modyanov N, Malhotra D, Haller ST, Kennedy DJ. Harmful Algal Bloom Toxicity in *Lithobates catesbeiana* Tadpoles. *Toxins*. 2020; 12(6):378.

Su RC, Warner EA, Breidenbach JD, **Lad A**, Blomquist TM, Kleinhenz AL, Modyanov N, Malhotra D, Kennedy DJ, Haller ST. CD40 Receptor Knockout Protects against Microcystin-LR (MC-LR) Prolongation and Exacerbation of Dextran Sulfate Sodium (DSS)-Induced Colitis. *Biomedicines*. 2020; 8(6):149.

Su RC, **Lad A**, Breidenbach JD, Kleinhenz AL, Modyanov N, Malhotra D, Haller ST, Kennedy DJ. Assessment of diagnostic biomarkers of liver injury in the setting of microcystin-LR (MC-LR) hepatotoxicity, Chemosphere, Volume 257, 2020.

Baliu-Rodriguez D, Kucheriavaia D, Palagama DSW, **Lad A**, O'Neill GM, Birbeck JA, Kennedy DJ, Haller ST, Westrick J, Isailovic D. Development and Application of Extraction Methods for LC-MS Quantification of Microcystins in Liver Tissue. *Toxins*. 2020.

Zhang S, Breidenbach JD, Khalaf FK, Dube P, Mohammed CJ, **Lad A**, Stepkowski S, Hinds TJ, Kumarasamy S, Kleinhenz AL, Tian J, Malhotra D, Kennedy DJ, Cooper C, Haller ST. Renal Fibrosis is Significantly Attenuated Following Targeted Disruption of Cd40 in Experimental Renal Ischemia. *Journal of the American Heart Association*. 2020, 9(7): e014072.

Su RC, **Lad A**, Breidenbach JD, Blomquist TM, Gunning WT, Dube P, Kleinhenz AL, Malhotra D, Haller ST, Kennedy DJ. Hyperglycemia induces key genetic and phenotypic changes in human liver epithelial HepG2 cells which parallel the $Lepr^{db}$/J mouse model of non-alcoholic fatty liver disease (NAFLD). *PLoS One*. 2019, 14(12): e0225604.

Lad A, Su R, Breidenbach J, Stemmer P, Carruthers N, Sanchez N, Khalaf FK, Zhang S, Kleinhenz A, Dube P, Mohammed CJ, Westrick J, Crawford E, Palagama D, Baliu-Rodriguez D, Isailovic D, Levison BS, Modyanov N, Gohara A, Malhotra D, Haller ST, Kennedy DJ. Chronic Low Dose Oral Exposure to Microcystin-LR Exacerbates Hepatic Injury in a Murine Model of Non-Alcoholic Fatty Liver Disease. *Toxins*. 2019, 11(9), 486.

Su R, Blomquist T, Kleinhenz A, Khalaf F, Dube P, **Lad A**, Breidenbach J, Mohammed C, Shang S, Baum C, Malhotra D, Kennedy DJ, Haller ST. Exposure to the Harmful Algal Bloom (HAB) toxin microcystin-LR (MC-LR) prolongs and increases severity of dextran sulfate sodium (DSS)-induced colitis. *Toxins*. 2019, 11(6), 371.

Palagama DSW, Baliu-Rodriguez D, **Lad A**, Levison BS, Kennedy DJ, Haller ST, Westrick J, Hensley K, Isailovic D. Development and applications of solid-phase extraction and liquid chromatography-mass spectrometry methods for quantification of microcystins in urine, plasma, and serum. *J Chromatography A*. 2018; 1573:66-77.

Method for detection of antibodies against the ADDA region of Microcystins/ Nodularins in blood of exposed individuals. The University of Toledo College of Medicine, U.S. Kennedy DJ, Haller ST, Mukundan D, Wooten M, **Lad A**, Presloid J, Levison B, Huntley J. Application no.: 59990-US-NP/D2018-46, **Patent Filed** U.S. Patent No. 11,125,750

PRESENTED ABSTRACTS

Lad A, Presloid J, Wooten M, Mukundan D, Huntley J, Kennedy DJ, Haller ST. The Harmful Algal Bloom Toxin Microcystin-LR Induces an Antibody Response: Implications for Monitoring Exposure. *J Investig Med* 2021;69:1069 –1143; doi:10.1136/jim-2021-MW.76

Lad A, Hunyadi J, Connolly J, Khalaf FK, Dube P, Zhang S, Kleinhenz A, Baliu-Rodriguez D, Isailovic D, Malhotra D, Haller ST, Kennedy DJ. Antioxidant Therapy Restores Hepatic Phase I & Phase II Metabolic Enzymes Altered by Exposure to Microcystin-LR in a Murine Model of Diet Induced Non-Alcoholic Fatty Liver Disease. *J Investig Med* 2021;69:1069–1143; doi:10.1136/jim-2021-MW.79

Lad A, Kleinhenz A, Breidenbach JD, Khalaf FK, Dube P, Zhang S, Su R, Hunyadi J Hinds T, Baliu-Rodriguez D, Isailovic D, Connolly J, Khatib-Shahidi BZ, Malhotra D, Haller ST, Kennedy DJ. Antioxidant therapy significantly attenuates hepatotoxicity following low dose exposure to microcystin-LR in a murine model of diet-induced non-alcoholic fatty liver disease. Abstract published in *Journal of Investigative Medicine*, 2020; 68(5):1121

Lad A, Su R, Breidenbach J, Carruthers N, Stemmer P, Palagama D, Baliu-Rodriguez D, Isailovic D, Kleinhenz A, Khalaf FK, Zhang S, Dube P, Mohammed C, Gohara A, Malhotra D, Haller ST and Kennedy DJ. Hepatic injury associated with reduced urinary excretion and altered hepatic signaling pathways following chronic low dose exposure to microcystin-LR in a murine model of non-alcoholic fatty liver disease. Poster presentation at Harmful Algal Blooms Conference, Toledo, OH, Sep 12, 2019

Lad A, Su R, Breidenbach J, Carruthers N, Stemmer P, Palagama D, Baliu-Rodriguez D, Isailovic D, Kleinhenz A, Khalaf FK, Zhang S, Dube P, Mohammed C, Gohara A, Malhotra D, Haller ST and Kennedy DJ. Hepatic injury associated with reduced urinary excretion and altered hepatic signaling pathways following chronic low dose exposure to microcystin-LR in a murine model of non-alcoholic fatty liver disease. Poster presentation at Midwest Clinical and Translational Research Meeting; April 4-5, 2019, Chicago, IL 2019

Lad A, Su R, Breidenbach J, Carruthers N, Stemmer P, Palagama D, Baliu-Rodriguez D, Isailovic D, Kleinhenz A, Khalaf FK, Zhang S, Dube P, Mohammed C, Gohara A, Malhotra D, Haller ST and Kennedy DJ. Hepatic injury associated with reduced urinary excretion and altered hepatic signaling pathways following chronic low dose exposure to microcystin-LR in a murine model of non-alcoholic fatty liver disease. Poster presentation at Graduate Research Forum, University of Toledo, OH, Mar 19-21, 2019.

Lad A, Spegele A, Kleinhenz A, Khalaf FK, Zhang S, Su R, Malhotra D, Haller ST, and Kennedy DJ. Evidence of hepatotoxicity following chronic low dose exposure to microcystin-LR in a murine model of non-alcoholic fatty liver disease. Poster presentation at Harmful Algal Blooms Conference, Toledo, OH, Sep 13, 2018

Lad A, Spegele A, Kleinhenz A, Khalaf FK, Zhang S, Su R, Malhotra D, Haller ST, and Kennedy DJ. Evidence of hepatotoxicity following chronic low dose exposure to microcystin-LR in a murine model of non-alcoholic fatty liver disease. Poster presentation at Pharmacology Colloquium, Michigan State University, MI June 1, 2018

Lad A, Spegele A, Kleinhenz A, Khalaf FK, Zhang S, Su R, Malhotra D, Haller ST, and Kennedy DJ. Evidence of hepatotoxicity following chronic low dose exposure to microcystin-LR in a murine model of non-alcoholic fatty liver disease. Published abstract in *Journal of Investigative Medicine*, Vol. 66, No. 4, pp. 824-824 and Oral presentation at Midwest Clinical and Translational Research Meeting; April 26-27, 2018, Chicago, IL 2018

Lad A, Spegele A, Kleinhenz A, Khalaf FK, Zhang S, Su R, Malhotra D, Haller ST, and Kennedy DJ. Evidence of hepatotoxicity following chronic low dose exposure to microcystin-LR in a murine model of non-alcoholic fatty liver disease. Poster presentation at Graduate Research Forum, University of Toledo, Toledo, OH Mar 15-16, 2018

Lad A, Sanchez N and Kennedy DJ, Effect of Pre-Existing Liver Disease on Susceptibility to Microcystin Induced Toxicity, Oral presentation at Lake Erie Center, National Science Foundation Research Experience for Undergraduates, Toledo, OH, May 05, 2017