Dihydromyricetin (DHM) increases lipid clearance and mitochondrial function in mice exposed to long-term ethanol consumption

Karina Hiranandani, Eva Jones, Jinah Kim, Samantha G. Skinner, Dr. Isis Janilkarn-Urena, Dr. Daryl Davies

Dept of X, Bridge Institute, University of Southern California, Los Angeles, CA , USA

Bridge UnderGrad Science (BUGS) ***Summer Research Program***

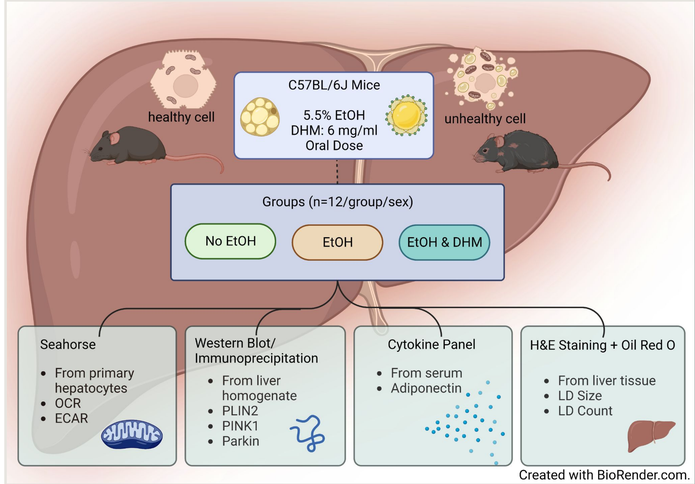
USC University of Southern California

Introduction

Alcohol-associated liver disease (ALD) is one of the most prevalent liver diseases in the United States. Approximately 140,000 people die from alcohol-related causes annually, making it one of the top five causes of death in the country.[1] ALD is characterized by three major stages, progressing from steatosis to steatohepatitis/fibrosis, to finally cirrhosis, an end-stage liver disease. Unfortunately, there are no effective treatments for ALD besides abstinence, or in extreme cases, liver transplant. Corticosteroids are used for the treatment of inflammation, but long-term use is associated with adverse side effects and risk of infection.[2] Alcohol consumption has extensive negative effects on bodily processes. Increased ethanol (EtOH) metabolism results in the dysregulation of energy signaling and lipid metabolic pathways through an elevation in reactive oxygen species (ROS) and acetaldehyde.[3] Additionally, chronic alcohol intake alters mitochondrial function via impairment of oxidative phosphorylation leading to decreased oxidative metabolism, ATP synthesis, and hepatocyte tolerance to stress.[4] Mitochondrial proliferation and increased activity of critical enzymes involved in mitophagy can regulate healthy mitochondria function. Dihydromyricetin (DHM) is a flavonoid derived from many plants, including *Hovenia dulcis*. DHM has been utilized as an anti-alcohol therapeutic in Traditional Chinese Medicine for over 600 years and is sold over the counter today as a "hangover cure."[5] Promising evidence shows that DHM has hepatoprotective effects via the regulation of bioenergetics, activation of lipid oxidation pathways, and reduction of oxidative stress.[6]

Methods

Female and male C57BL/6J mice (n=12/group/sex) were treated with an isocaloric ethanol-containing Lieber-DeCarli diet, a widely used ad libitum drinking model that closely mimics ALD pathology. Mice were randomly assigned to three groups: 1) No-EtOH, 2) EtOH [5.5% (v/v)], 3) EtOH [5.5% (v/v)] + DHM (6mg/mL). Mice receiving EtOH + DHM were administered ethanol-only for 2 weeks prior to DHM supplementation to establish the development of ALD pathology. Protein expression of PLIN1 was quantified from immunoprecipitation while PINK1, Parkin, and PLIN2 were quantified from Western Blot; all protein was isolated from liver homogenate. Immunohistochemistry was performed on liver tissue using H&E and Oil Red O staining. Adiponectin was measured from serum using a cytokine panel. Primary hepatocytes were isolated to measure oxygen consumption rate and extracellular acidification rate using a Seahorse analyzer. Data is presented as mean ± standard deviation. Statistical analysis included 2-way ANOVA along with Bonferroni multiple comparison tests using Prism 8.3 (GraphPad Software, Inc, CA). $p \leq 0.05$ was considered statistically significant.



Results

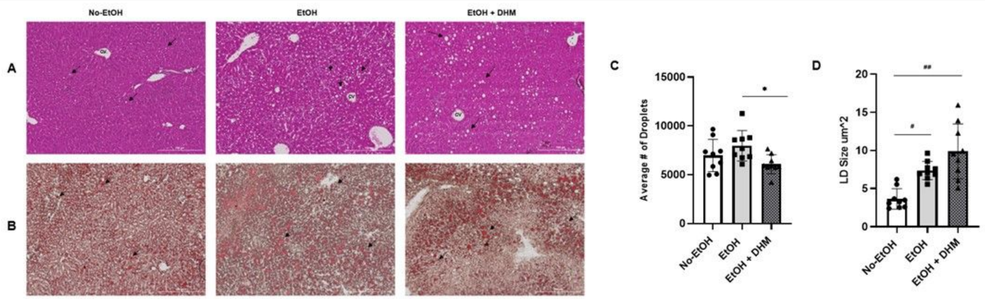


Figure 1: DHM administration increased lipid droplet (LD) size and decreased LD count as compared to the EtOH-treatment group. Hematoxylin and Eosin (H&E) staining (A) and Oil Red O staining (B), were employed to visualize liver tissue. The H&E images showed vacuoles in white, whereas Oil Red O stained LDs bright red, as signified by black arrows. The findings revealed that DHM supplementation (C) decreased quantity of lipid droplets (*0.025) and (D) increased size as compared to the EtOH-treatment group (#0.0063, ##<0.0001).

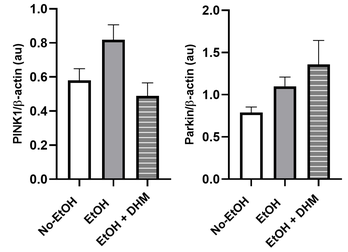


Figure 2: DHM administration improved the expression of mitophagy-related proteins. Uncleaved PINK1 assembles on the outer mitochondrial membrane of damaged mitochondria where it can be cleaved/activated and is one of the precursors to mitophagy. Parkin is recruited by PINK1 to mark damaged mitochondria for degradation. (A) DHM-supplementation group shows the lowest levels of uncleaved PINK1. (B) Parkin protein expression in the DHM-supplementation group is increased.

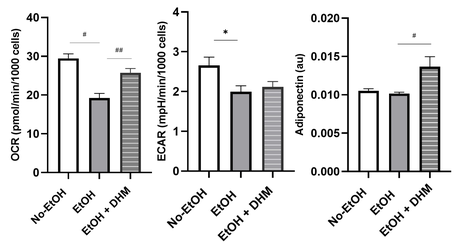


Figure 3: **DHM supplementation leads to improved mitochondrial-driven respiration.** (A) Oxygen consumption rate (OCR) represents mitochondrial aerobic respiration and is significantly increased in the DHM-fed group when compared to the EtOH-only group (#<0.0001, ## 0.0003); (B) Extracellular acidification rate (ECAR) represents glycolysis-driven metabolism (*:0.0211); and (C) Adiponectin is a cytokine that has been shown to promote β-oxidation. Adiponectin levels were significantly increased in the DHM-fed group (#0.0431).

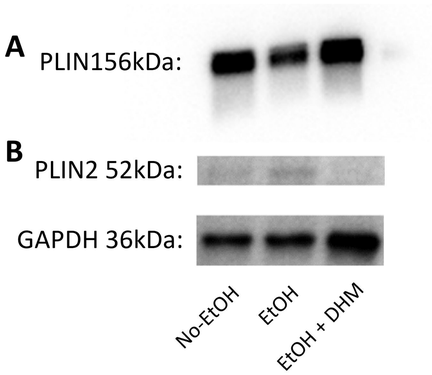


Figure 4: DHM supplementation alters perilipin composition in LD membranes. Perilipins are expressed on LD membranes and are involved in regulating their maintenance and degradation. (A) Immunoprecipitation data of PLIN1 demonstrates the highest protein expression in the DHM group. Increased PLIN2 expression has been shown to protect LDs from autophagy. (B) PLIN2 levels were notably lower in DHM-supplementation models.

Discussion

The prevalence of ALD and the lack of FDA-approved therapies necessitates the development of effective therapeutics. Given the hepatoprotective benefits of DHM, the present study investigated the effects of DHM supplementation on lipid accumulation and mitochondrial health in hepatic tissue. We observed an increase in lipid droplet (LD) heterogeneity and clearance with DHM administration. Additionally, DHM appeared to alter the membrane composition of LDs, indicating a shift toward larger LDs. This preliminary data suggests that DHM influences accumulation, thereby aiding in LD clearance. DHM-fed mice also show improved mitochondrial health and function, efficiency of oxidative phosphorylation, and β-oxidation as shown through improved OCR, ECAR, and adiponectin levels. Additionally, results measuring mitophagy-associated proteins, PINK1 and Parkin, indicate that DHM aids in the removal of damaged mitochondria, specifically through a process called mitophagy. Mitochondrial homeostasis is regulated by mitophagy which allows for increased beta-oxidation and removal of LDs, a necessary process in preventing and treating steatosis.[7] Potential future areas of study include DHM-induced lipid morphology and lipophagy, and the pathway by which EtOH and DHM affect PINK1 expression in dysfunctional mitochondria. Taken together, this data demonstrates that DHM positively modulates mitochondrial activity and LD accumulation. Mitochondrial damage and lipid accumulation are major causes of ALD progression. This evidence will support future research investigating DHM as a promising therapeutic to treat ALD by providing insight into DHM's role in mitigating mitochondrial damage and hepatic lipid accumulation.

References

1. National Institute on Alcohol Abuse and Alcoholism. Alcohol-Related Emergencies and Deaths in the United States. NIAAA. 2023.
1. Saberi, B.; Dadabhai, A.S.; Jang, Y.Y.; Gurakar, A.; Mezey, E. Current Management of Alcoholic Hepatitis and Future Therapies. J Clin Transl Hepatol. 2016 Jun 28;4(2):113-22.
2. Wu, Y.C.; Yao, Y.; Tao, L.S.; Wang, S.H.; Hu, Y.; Li, L.Y.; Hu, S.; Meng, X.; Yang, D.S.; Li, H.; Xu, T. The role of acetaldehyde dehydrogenase 2 in the pathogenesis of liver diseases, Cellular Signaling, Volume 102, 2023, 110550.
3. Grattagliano, I.; Russmann, S.; Diogo, C.; Bonfrate, L.; Oliveira, P.J.; Wang, D.Q.; Portincasa, P. Mitochondria in chronic liver disease. Curr Drug Targets. 2011 Jun;12(6):879-93.
4. Carneiro, R.C; Ye, L.; Baek, N.; Teixeira, G.H.; O'Keefe, S.F. Vine tea (Ampelopsis grossedentata): A review of chemical composition, functional properties, and potential food applications, Journal of Functional Foods, Volume 76, 2021, 104317.
5. Silva, J.; Spatz, M.H.; Folk, C.; Chang, A.; Cadenas, E.; Liang, J.; Davies, D.L. Dihydromyricetin improves mitochondrial outcomes in the liver of alcohol-fed mice via the AMPK/Sirt-1/PGC-1α signaling axis. Alcohol. 2021 Mar; 91:1-9.
6. Williams, J., Ding, W., Role of autophagy in alcohol and drug-induced liver injury, Food and Chemical Toxicology, Volume 136, 2020, 111075.

Acknowledgements

We would like to thank Mindy Zhang and the Bridge UnderGrad Science (BUGS) Summer Research Program for their contributions to this project.

Funding provided by: The National Institute on Alcohol Abuse and Alcoholism (NIAAA), and in part by Timothy M. Chan Professorship in Complementary Therapeutics (USC) and the USC Institute of Addiction Science (IAS).

CONTACT US

bridge.usc.edu/bugs