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Dihydromyricetin Ameliorates Inflammatory Markers in Mice with Alcohol-Associated Fatty Liver Disease

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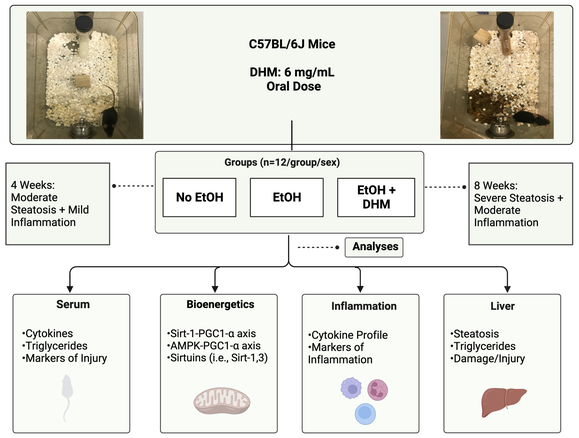
INTRODUCTION

- In the United States alone, Alcohol-associated Liver Disease (ALD) is becoming increasingly prevalent and currently affecting over 28 million people.
- Alcohol-associated fatty liver disease (AFLD) and alcohol-associated steatohepatitis (ASH) are the early stages of ALD that are reversible through abstinence in most individuals.
- Meanwhile, liver fibrosis and cirrhosis are reached in later stages and, unfortunately, irreversible.
- Progression of ALD into stages such as ASH are dependent on progression of inflammation in the body.
- Dihydromyricetin (DHM), a bioactive polyphenol and flavonoid extracted from many plants including Hovenia dulcis, has previously exhibited hepatoprotective and anti-inflammatory effects through intraperitoneal injection (i.p.).
- In the present study, we tested the effects of DHM on inflammatory markers associated with ALD pathology in female mice using an ALD model, the Lieber DeCarli (LDC) diet.

METHODS

Female c57BL/6J mice (n=12/group) were treated using a forced-drinking paradigm, the Lieber DeCarli ethanol-containing liquid diet for 5 weeks. Mice were randomly divided into three groups: 1) No-EtOH, 2) EtOH [5% (v/v)], 3) EtOH [5% (v/v)] + DHM (6mg/mL).
All groups were isocaloric. Mice in the EtOH and EtOH + DHM groups were administered ethanol for 2 weeks in order to ensure ALD pathology development. Subsequently, DHM supplementation in the EtOH + DHM group started and continued until the end of the study. Statistical analysis included one-way ANOVA along with Bonferroni multiple comparison tests using Prism 9.3 (GraphPad Software, Inc., San Diego, CA), where $p \leq 0.05$ was considered statistically significant.

ALD Model: Lieber DeCarli (LDC) Diet



RESULTS

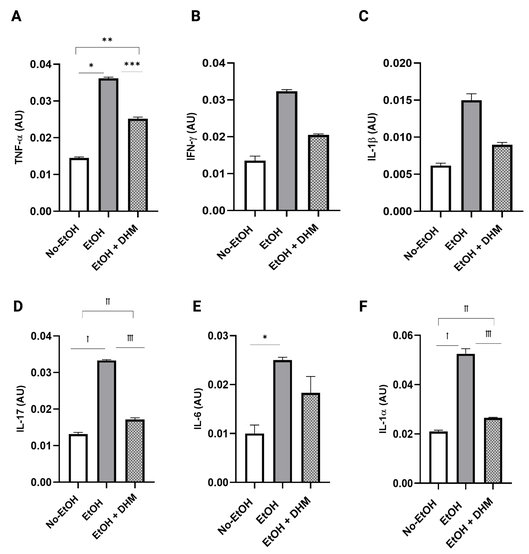


Figure 1. **(A)** DHM-fed mice show significant decreases in levels of TNF-α compared to EtOH-only mice (*,**,*** <0.0001). Normalization of levels of **(B)** IFN- γ and **(C)** IL-1β to those similar to those in the No-EtOH group is seen. DHM-fed mice show significant decreases in **(D)** IL-17 (ᵗ <0.0001, ᵗᵗ0.003, ᵗᵗᵗ <0.0001) compared to EtOH-only mice. EtOH-only mice show a significant increase in **(E)** IL-6 (*0.007) expression compared to No-EtOH mice. **(F)** IL-1α (ᵗ <0.0001, ᵗᵗ0.047, ᵗᵗᵗ <0.0001) levels are significantly reduced in DHM-fed mice compared to EtOH-only mice.

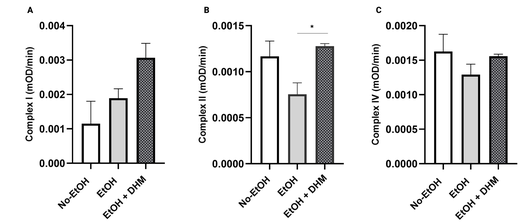


Figure 2. DHM-fed mice demonstrate improvement in mitochondrial oxidative phosphorylation systems as shown by restored activity in **(A)** complex I, **(B)** complex II (*0.03), and **(C)** complex IV.

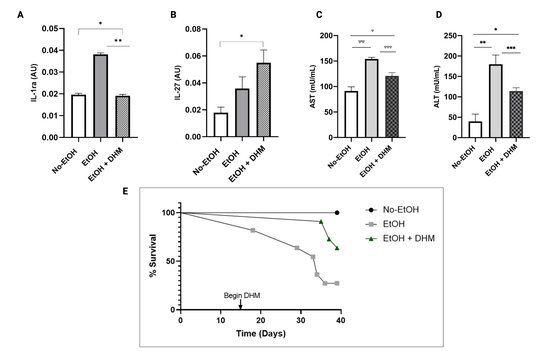
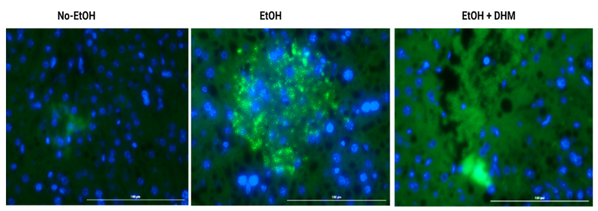


Figure 3. Expression of **(A)** IL-1ra (*,**<0.0001) is significantly reduced in mice receiving DHM compared to those fed EtOH-only. In addition, levels of circulating **(B)** IL-27 (*0.034) in the DHM-fed group are significantly higher than that in the No-EtOH group. Circulating **(C)** aspartate aminotransferase (AST) levels (*0. 029, ᵛᵛ0.0006, ᵛᵛᵛ0.024) and **(D)** alanine aminotransferase (ALT) levels (*0.027, **0.0014, ***0.0046) are reduced with DHM administration. **(E)** DHM administration enhanced the survival rate of the mice in the study.



CD68 (Stains for Macrophages)

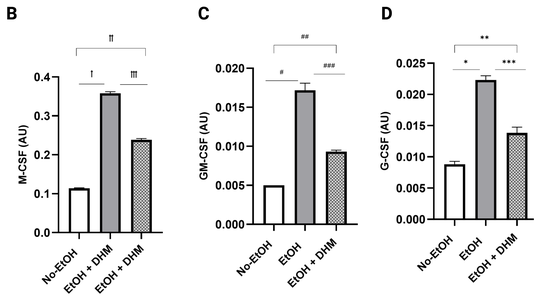


Figure 4. **(A)** Liver sections stained with known macrophage stain CD68 (green) indicate that EtOH-only group mice have larger macrophage infiltration compared to the No-EtOH and DHM-fed mice. Expression of **(B)** macrophage-colony stimulating factor (M-CSF; ᵗ,ᵗᵗ,ᵗᵗᵗ <0.0001), **(C)** granulocyte-macrophage-colony stimulating factor (GM-CSF; #<0.0001, ## 0.003, ###0.0001), and **(D)** granulocyte-colony stimulating factor (G-CSF; *<0.0001, **0.005, ***0.0004) significantly decreases with DHM administration.

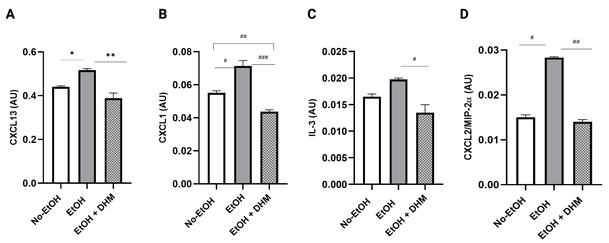


Figure 5. Expression of chemokines **(A)** CXCL13 (*0.023, **0.002) and **(B)** CXCL1 (#<0.0001, ##0.0001, ###<0.0001) is also reduced in DHM-fed mice. Expression of pro-inflammatory cytokine **(C)** IL-3 (*0.035) and pro-inflammatory chemokine **(D)** CXCL2 (#,##<0.0001) is reduced following DHM administration.

CONCLUSION

Alcohol sales and rates of alcohol-related morbidity continue to grow despite the detrimental effects of alcohol consumption. Chronic ethanol consumption negatively affects the body in many ways, including declining liver function and decreased mitochondrial function. There is still no approved pharmaceutical or nutritional supplement to ameliorate ALD. With the increasing public interest in natural and herbal therapies containing polyphenols and flavonoids, DHM has become the focus of several studies investigating hepatoprotective effects[1] including inflammation[2]. The present study tested the hypothesis that DHM can mediate the levels of pro- and anti-inflammatory cytokines and therefore, ameliorate inflammatory damages associated with ALD.

Our results demonstrate the ameliorative effects of DHM on hepatic function as shown with significantly reduced circulating AST and ALT levels compared to those seen in the EtOH-only group. Reactive oxygen species (ROS) accumulation, such as from ethanol breakdown in the mitochondria, can reduce mitochondrial function as well as induce inflammation. DHM-fed mice show improvement in oxidative phosphorylation systems which point to improved mitochondrial health and function. Our results show a significant increase in Complex II activity in DHM-fed mice compared to that seen in EtOH-only mice. As presented here, mice receiving DHM show reduced pro-inflammatory cytokine levels such as TNF-α, IFN-γ, and IL-1β which are elevated in ALD patients[3]. DHM-fed mice have increased anti-inflammatory cytokines, specifically, DHM led to a significant increase in IL-27, a cytokine known for protective action on the gut barrier, regenerative activity in the liver and intestines, and promoting intestinal barrier repair post ethanol injury[4].

Overall, DHM administration shows modulation of inflammatory cytokines and amelioration of some of the detrimental effects of chronic ethanol consumption. DHM's action on ALD progression is likely multifactorial with many more complexities expected to be uncovered in future studies.

REFERENCES

1. Silva J, Spatz MH, Folk C, Chang A, Cadenas E, Liang J, Davies DL. 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. doi: 10.1016/j.alcohol.2020.10.002. Epub 2020 Oct 17. PMID: 33080338; PMCID: PMC7902334.
2. Sun Y, Liu S, Yang S, Chen C, Yang Y, Lin M, Liu C, Wang W, Zhou X, Ai Q, Wang W, Chen N. Mechanism of Dihydromyricetin on Inflammatory Diseases. Front Pharmacol. 2022 Jan 18;12:794563. doi: 10.3389/fphar.2021.794563. PMID: 35115939; PMCID: PMC8804380.
3. Kawaratani H, Tsujimoto T, Douhara A, Takaya H, Moriya K, Namisaki T, Noguchi R, Yoshiji H, Fujimoto M, Fukui H. The effect of inflammatory cytokines in alcoholic liver disease. Mediators Inflamm. 2013;2013:495156. doi: 10.1155/2013/495156. Epub 2013 Dec 9. PMID: 24385684; PMCID: PMC3872233.
4. Luck ME, Li X, Herrnreiter CJ, Cannon AR, Choudhry MA. IL-27 Promotes Intestinal Barrier Integrity following Ethanol Intoxication and Burn Injury. Immunohorizons. 2022 Aug 16;6(8):600-613. doi: 10.4049/immunohorizons.2200032. PMID: 35973751.

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