

The Effects of Dihydromyricetin on Bile Acid Levels in Mice Exposed to Chronic Ethanol Consumption

Sarah Chen, Ky Duong, Julio Rivera, Samantha Skinner, Dr. Isis Janilkarn-Urena, Dr. Daryl Davies

USC Alfred E Mann School of Pharmacy and Pharmaceutical Sciences, Bridge Institute, University of Southern California, Los Angeles, CA, USA



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Abstract

Alcohol-associated liver disease (ALD) is one of the major causes of liver failure and death worldwide. ALD is mainly caused by chronic heavy alcohol consumption, leading to a range of liver injuries from steatosis to more severe cases of cirrhosis. While the liver is the primary site for alcohol metabolism, it is also the site of bile acid production. Bile acids are secreted to the small intestine to emulsify dietary fats and vitamins, which are absorbed and transported back to the liver for metabolism and distribution. Cholestasis is a condition characterized by an imbalance of bile acid transport and is also heavily associated with ALD. Farnesoid X receptor (FXR), the master regulator of bile acid production, is primarily expressed in the liver and intestines. Evidence suggests that alcohol interferes with FXR activation, which in turn affects bile acid homeostasis and the progression of ALD. Dihydromyricetin (DHM) is a bioflavonoid extracted from *Hovenia dulcis* that is implicated in bile acid synthesis and transport. Here, we explored the effects of DHM on FXR in mice treated with a chronic alcohol consumption model. We find that DHM increases FXR activity, total bile acid levels, and increased total esterified cholesterol levels, while decreasing hepatic TG, and altering fecal bacteria content. Additionally, DHM was found to increase survival rates and per Western Blot (WB) analysis, enhanced expression of Farnesoid-X receptor (FXR), a master regulator of bile acid homeostasis that also plays a major role in lipid metabolism. Current studies suggest there is a strong link between farnesoid X receptor (FXR) and bile acids and their roles in the development of ALD.

Objectives

- The objective of this project is to investigate the effect of oral DHM on the gut by way of investigating bile acid production and changes in the microbiome.
- The project measured fecal microbiota composition, bile acid levels, and FXR protein expression.

Methods

To study the effect of DHM on bile acid synthesis, a Lieber-DeCarli (LDC) diet was administered to male and female C57BL/6 mice. The LDC diet is a widely utilized and accepted experimental model of ALD in rodents. Mice were randomly assigned into three groups (n=12/group/sex): 1) No-EtOH 2) EtOH (3.6% v/v) and 3) EtOH (3.6% v/v) + DHM (6 mg/mL). Treatment lasted 5 weeks. Mice in the DHM supplementation group were fed EtOH-only for two weeks prior to DHM to ensure disease progression. Protein expression of FXR isolated from liver homogenates was quantified via immunoprecipitation and visualized via SDS-PAGE, biochemical assays were utilized to measure circulating bile acid and esterified cholesterol levels, and fecal bacteria assays were conducted for each of the experimental groups. Data is presented as mean \pm standard deviation and 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

Bile Acid and Esterified Cholesterol Levels

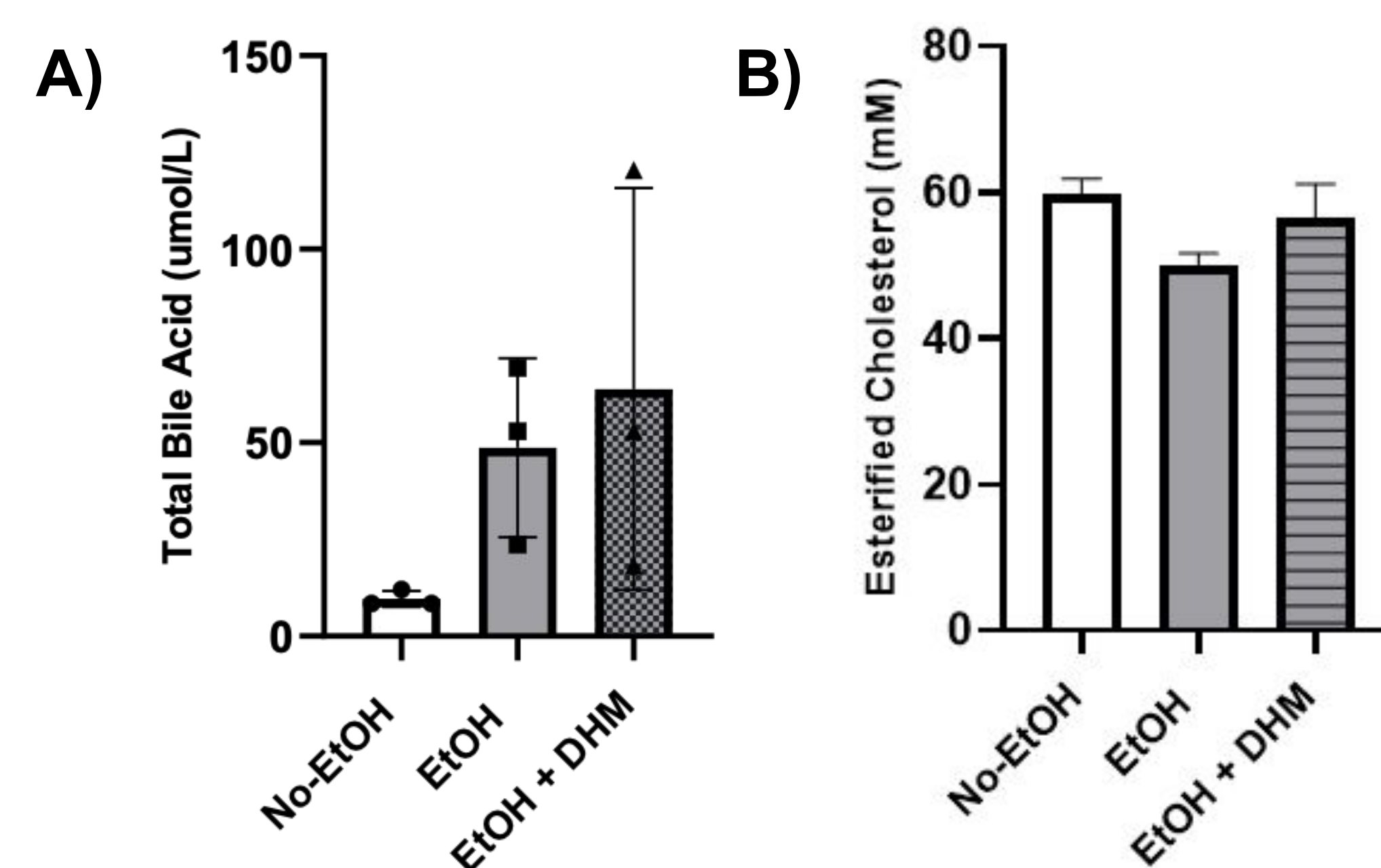


Figure 1. A) DHM Administration Increases Total Bile Acid Levels. Bile acids are secreted from the gallbladder to the intestines via enterohepatic circulation to aid in the solubilization and absorption of dietary fats. Alcohol has been shown to increase the total bile acid pool. Cholestasis is a condition in which this bile acid flow is disrupted, ultimately leading to a decrease in circulating bile acids and an accumulation of total bile acids in the gallbladder. Total circulating bile acid levels were measured after the 6-week treatment period demonstrating a trend of increased bile acid production.

Changes to Bacterial Populations



Figure 2. Changes in bacterial populations in female mice. Different bacterial populations found in the gut microbiota influence absorption and metabolism of dietary nutrients, including the production of secondary bile acids. Populations of bacterial species were investigated and presented in **A)** No EtOH, **B)** EtOH, and **C)** EtOH + DHM groups. Notably, certain species of bacteria known to metabolize, hydrolyze, and conjugate bile acids—*Adlercreutzia*, *Hungatella*, and *B. globosum*—were most abundant in the DHM group.

Fecal Bacteria Content

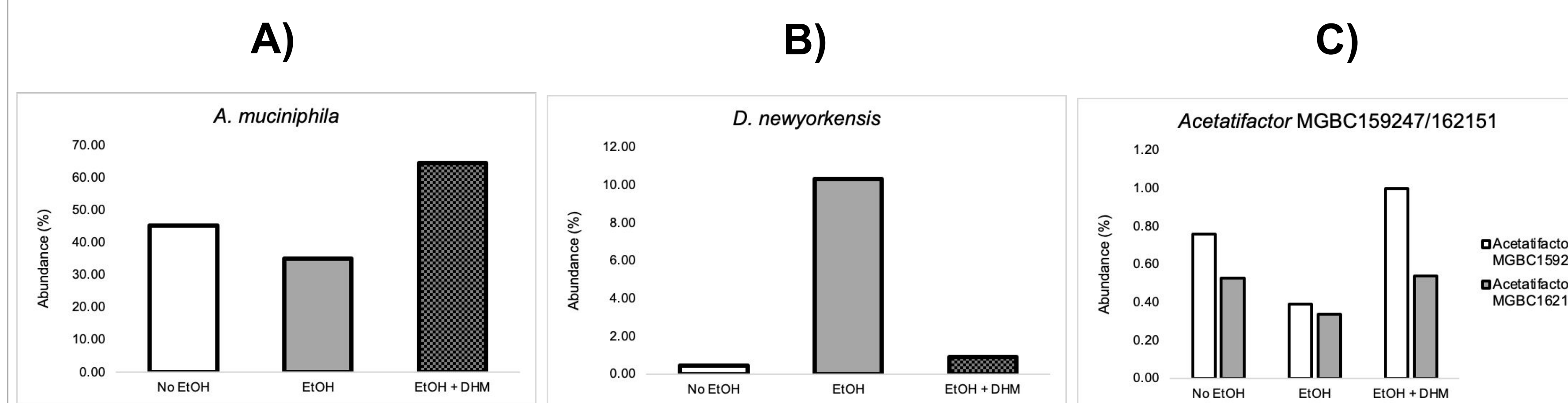


Figure 3. DHM Administration reveals positive changes in bacterial population involved in gut function and bile acid modification. **A)** *A. muciniphila*, involved in gut barrier function and mucus production, is disrupted by EtOH, but increased with DHM. **B)** *D. newyorkensis*, involved with gut endothelial function and reducing oxidative stress, is normalized with DHM. **C)** *Acetatifactor*, known to deconjugate bile acids, is diminished by EtOH, but restored with DHM.

FXR Protein Expression



Figure 4. DHM Administration Upregulates FXR Expression. Farnesoid X receptor (FXR) is a bile acid-induced transcription factor that plays an important regulatory role in bile acid synthesis. Differences in immunoprecipitated protein expression of FXR isolated from liver homogenates are shown. EtOH + DHM mice exhibited higher levels of FXR expression compared to No EtOH and EtOH-only mice.

Conclusion

ALD remains a leading cause of liver failure and mortality worldwide, making it imperative to develop an effective FDA-approved therapeutic that addresses this disease burden. Alcohol consumption disrupts multiple organ systems, including the gut. The ALD model used in this study is known to mimic clinical pathology commonly associated with ALD development and progression. One of these includes the dysregulation of bile acids, such as what is observed with cholestasis. Research conducted by others has shown that chronic alcohol consumption may lead to bile acid dysregulation and thus exacerbate ALD¹. Normally, primary bile acids are synthesized in the liver and secreted into bile. They are then stored in the gallbladder and released into the small intestine following a meal to aid in the digestion and absorption of dietary fats. In this study, we observed that total circulating bile acid levels were increased in the DHM-treated group. Reverse cholesterol transport is a process through which excess cholesterol is removed from circulation and peripheral adipose tissues, esterified, and transported back to the liver to undergo one of three main fates, namely conversion into bile acids. DHM supplementation appeared to normalize levels of esterified cholesterol. In addition to the bile acid population, we looked at intestinal bacteria populations as they are known to biotransform primary bile acids into secondary bile acids. Ethanol consumption is known to disrupt the gut microbiomes function, impacting bile acid homeostasis². DHM-fed mice had increased populations of bacteria that are known to metabolize, hydrolyze, and conjugate bile acids, producing secondary bile acids^{3,4}. Research has shown that disruption of FXR activity via chronic alcohol consumption may lead to bile acid dysregulation and thus exacerbate ALD, suggesting the potential of FXR as a target in the reversal of ALD¹. Immunoprecipitated protein expression of FXR showed higher levels of FXR expression in mice receiving DHM supplementation. Taken together, this preliminary study suggests that DHM may have a positive effect on FXR-driven activity leading to bile acid synthesis and eventually lipid transport. Further investigation is required to fully elucidate the relationship between chronic alcohol consumption, bile acids, and the gut microbiota.

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CONTACT US

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