Effect of Oral Dihydromyricetin (DHM) on EtOH-Induced Lipid Accumulation in Alcohol-Associated Liver Disease (ALD)

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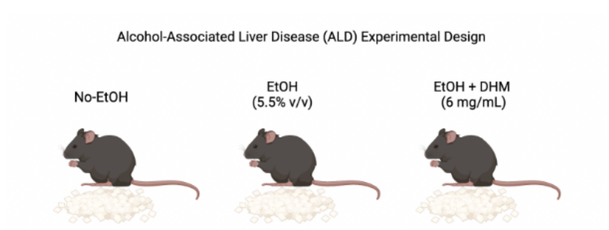
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INTRODUCTION

Alcohol-Associated liver disease (ALD) is caused by excessive consumption of ethanol (EtOH) and is one of the leading causes of death in the United States[1]. As the liver metabolizes EtOH, toxins are formed that cause liver injury, lipid accumulation, and hepatocyte death[2]. Eventually, the disruption in lipid homeostasis and metabolism will lead to fatty liver disease (steatosis). ALD is a progressive disease characterized by the following stages: alcohol-induced fatty liver disease (AFLD; steatosis), alcohol-associated steatohepatitis/fibrosis (ASH), to ultimately, cirrhosis[3]. Steatosis is the early stage and is defined as the deposition of fat in hepatocytes that can progress to alcohol-associated steatohepatitis, which has greater inflammation injury. This stage of ALD can lead to the development of fibrosis[4]. Moreover, the fibrotic response may progress to cirrhosis, which is characterized by permanent liver scarring, hepatocyte, and mitochondrial damage that is irreversible[4]. Dihydromyricetin (DHM), a flavonoid extracted from many plants, including *Hovenia dulcis* has shown great potential in its hepatoprotective effects. DHM is known to reverse lipid accumulation, improve bioenergetics, and improve mitochondrial function[5]. However, as with most flavonoids, DHM has poor water solubility and low bioavailability when taken orally[6]. The goal of this study was to investigate the effects of a novel oral DHM formulation against EtOH-induced lipid accumulation using a widely used ALD animal model, the Lieber DeCarli liquid diet using female C57BL/6J mice[7].

METHODS

A forced drinking study was conducted using 6-8 week old female C57BL/6J mice (n=12/group), incorporating the Lieber DeCarli (LDC) liquid diet. Mice were individually housed and randomly placed into the following groups: 1. No-EtOH; 2. EtOH (5.5% v/v); and 3. EtOH + DHM (6 mg/mL). Mice were monitored daily for signs of morbidity, along with recordings of body weight and food intake. Mice were exposed to EtOH for a total of five weeks. The group receiving DHM was exposed to EtOH-only for 15 days prior to DHM administration to ensure the development of ALD pathology and received DHM-containing feed for the last three weeks of the study. Mice were euthanized via CO_2 exposure and confirmed by cervical dislocation. Blood and livers were harvested and stored at -80°C until use. Various biochemical assays were performed to measure levels of circulating triglycerides and measured liver health using aspartate and alanine aminotransferase (AST and ALT). In addition, protein expression was analyzed using immunohistochemistry (IHC), including Oil Red O to stain lipid droplets and antibodies against proteins of interest that are involved in maintaining lipid homeostasis and metabolism.



RESULTS

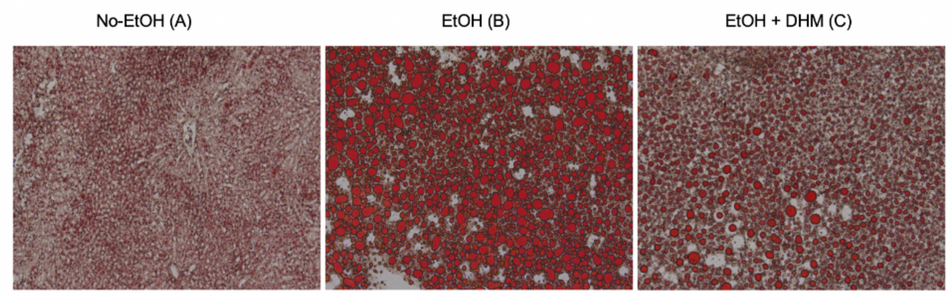


Figure 1. As illustrated, this is liver tissue sections stained with Oil Red O using 6-8 week old female C57BL/6J mice. The red lipid droplets are lipid vacuoles. There are three groups total: No-EtOH (A), EtOH (B), EtOH + DHM (C). The mice receiving No-EtOH displayed limited lipid droplets (LDs). The EtOH-only group displayed bigger LDs compared to the No-EtOH group. The mice receiving EtOH-DHM displayed larger lipid droplets compared to EtOH-only group. In addition, oral DHM in the EtOH-DHM group shows reduced number of LDs compared to EtOH-only group.

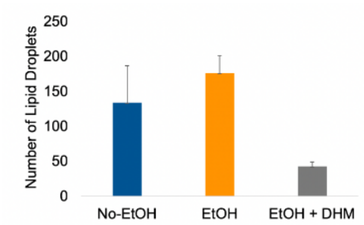


Figure 2. The graph illustrates the number of LDs in each group. Between the EtOH and EtOH + DHM groups, there is a decrease in the number of LDs. In comparison to the No-EtOH and EtOH + DHM groups, there was a decrease in the number of LDs.

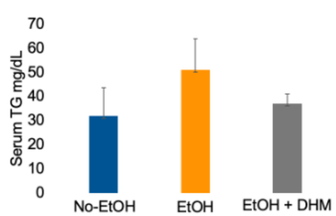


Figure 3. As illustrated, this is the level of serum triglyceride (TG). The EtOH + DHM group shows reduced levels of circulating TG compared to the EtOH-only group, in addition to reaching similar levels to the No-EtOH group.

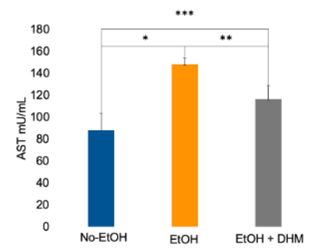


Figure 4. Aspartate aminotransferase (AST) has a major role in the metabolism of amino acids. The graph shows a significant decrease in AST levels in mice receiving oral DHM (*p=0.0002; **p=0.0261; ***p=0.0319). Higher levels of AST indicate liver damage.

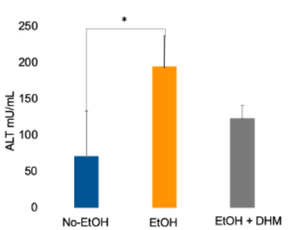


Figure 5. Alanine aminotransferase (ALT) is involved in protein conversion. The graph shows lower ALT levels in female mice receiving oral DHM. There was a significant decrease in ALT levels when comparing the No-EtOH and EtOH-only group (*p=0.0162). Higher levels of ALT indicate liver damage.

CONCLUSION

Overall, these results have shown that oral DHM have significant potential as an effective therapeutic for the consequential damage that results from excess EtOH-induced lipid accumulation in ALD. There were reduced amounts of lipid droplets, reduced levels of circulating triglycerides, reduced amount of lipid accumulation, and improved liver health and function via AST and ALT levels using a widely used ALD animal model.

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