Antialcohol Effects of Dihydromyricetin in Combination With Other Flavonoids

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Abstract
Herbal remedies are consumed by approximately 50% of the population in the United States for health and wellness, including products promoted for liver health and alcohol (ethanol [EtOH]). Previously, we have shown that dihydromyricetin (DHM), a bioflavonoid, can counteract EtOH intoxication and withdrawal via GABA<sub>A</sub> receptor (GABA<sub>A</sub>R) activity. Through evaluation of GABA<sub>A</sub>R potentiation using DHM, resveratrol, genistein, daidzein, and turmeric, we found that the activity of DHM is unique. Furthermore, using the loss of right reflex induced by EtOH in rats, we discovered that DHM was superior in reducing EtOH intoxication and EtOH actions on GABA<sub>A</sub>Rs. However, the combination of DHM with turmeric, daidzein, or resveratrol diminished the DHM effects. Here, we report that combinations of DHM should be evaluated, as we have found that combining DHM with other flavonoids diminished efficacy. Collectively, these data support the utility of DHM as a unique antialcohol intoxication therapy.

Keywords
ethanol, dihydromyricetin, bioflavonoids, LORR

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In the United States, approximately half the population consumes herbal remedies for the primary reason of promoting overall health and wellness. Notably, over the past decade, a growing focus of attention of flavonoid-based herbal medications is for the prevention of consequences associated with high alcohol (ethanol [EtOH]) intake, including reactive oxygen species (ROS) generation, liver injury, and hangover symptoms related to overindulgence. Moreover, as the trend continues, it appears that there is a tendency to combine several of these flavonoid/dietary compounds for the enhancement of the proposed phytochemical benefits. Interestingly, there is some suggestion that combining dietary supplements may diminish their efficacy. Therefore, the reduced activity of herbal supplements and flavonoid products may result in products that provide no benefit and may potentially result in severe clinical consequences. However, there remains a paucity of information regarding the potential loss of efficacy by these phytochemical combinations for the promotion of overall health. This lack of knowledge illustrates the need for additional studies before being proposed for consumption and marketed to the general population.

Previously, we have shown that dihydromyricetin (DHM) is effective as a positive allosteric modulator of GABA<sub>A</sub> receptors (GABA<sub>A</sub>R), has anxiolytic properties, improves cognition in Alzheimer’s disease (AD) in mice, enhances EtOH metabolism, and counteracts EtOH activity on GABA<sub>A</sub>Rs. The current study expands these investigations and illustrates how changes in the unique activity of DHM on GABA<sub>A</sub>Rs potentiation and its ability to counteract the inhibitory effects of EtOH can be affected. Additionally, we illustrate the importance of understanding the impact of combining dietary supplements—for improved or diminished outcomes.

Results and Discussion
DHM Significantly Counteracts EtOH Intoxication and EtOH-Mediated GABA<sub>A</sub>R Activity

Preclinical studies of DHM have reported therapeutic effects for several disorders, including chronic liver diseases, metabolic disorders, anxiety, and AD. In this

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For the first time, we systematically investigated the neuropharmacological properties of DHM as a positive modulator of GABA\textsubscript{A}Rs\textsuperscript{6,9} in the presence of other flavonoids. Here, we report that the activity of DHM on GABA\textsubscript{A}Rs is unique in potentiating GABA\textsubscript{A}Rs, counteracting EtOH effects on GABA\textsubscript{A}Rs, and reducing EtOH-mediated loss of right reflex (LORR) (Figure 1). To determine this, we compared the efficacy of DHM on GABA\textsubscript{A}R potentiation with other flavonoids (Figure 1(A)), which have been studied for the treatment of ROS protection, chronic liver diseases, anti-inflammation, and alcohol abuse.\textsuperscript{10,11} Our results suggest that DHM dose-dependently potentiated GABA\textsubscript{A}R-mediated I\textsubscript{tonic} (Figure 1(B), left) and miniature inhibitory postsynaptic currents (mIPSCs) (Figure 1(B), right; *$P < 0.001$, DHM doses vs dose 0; †$P < 0.001$, DHM vs other flavonoids) suggesting a unique activity of DHM versus the other flavonoids on GABA\textsubscript{A}R potentiation. Figure 1(C) illustrates the ability of various flavonoids to significantly reverse/reduce the action of EtOH on GABA\textsubscript{A}Rs. As depicted, EtOH (60 mM; E60) potentiated both I\textsubscript{tonic} (Figure 1(C), left) and mIPSCs (Figure 1(C), right). In the presence of EtOH (E60), DHM significantly reduced EtOH-induced enhancement of I\textsubscript{tonic} and mIPSCs, while other flavonoids showed no significant effects (*$P < 0.001$, drug vs drug 0. †$P < 0.001$, DHM vs other flavonoids).

To correlate these findings to behavioral responses associated with intoxication, we next assessed behavioral responses in LORR with drug administration. As presented in Figure 1(D), no administration of EtOH does not affect LORR in the saline group. As expected, the administration of EtOH influenced the LORR time, resulting in an average of about 73.6 ± 8.0 minutes. When investigating the respective treatments on EtOH-mediated LORR, we found that DHM significantly
reduced LORR compared with other flavonoid treatments (Figure 1(D); *P < 0.001). Furthermore, after 2 days of withdrawal (day 3), all animals received another dose of EtOH, including an administration of EtOH to the saline group. We found that the EtOH + DHM group showed minimal tolerance to EtOH compared with the saline group. In contrast, other groups had significantly reduced LORRs, suggesting increased tolerance to EtOH as measured on day 3 (*P < 0.001). Collectively, these results illustrate the significant benefit and potential of DHM compared with the other flavonoids tested as it pertains to its ability to potentiate GABA\(_A\)Rs, counteract EtOH activity on GABA\(_A\)Rs, and reduce EtOH intoxicating effects.

Therefore, the ability of DHM to potentiate GABA\(_A\)Rs provides a novel mechanism in its activity in comparison with other flavonoids that show similar benefits against EtOH intake and environmental stress. Therefore, the utility of DHM may be expanded to benefit EtOH abuse and withdrawal, and disorders mediated by GABA\(_A\)R signaling.

**DHM With Other Flavonoids Significantly Reduce DHM-Mediated GABA\(_A\)R Potentiation**

We also report that the activity of DHM on GABA\(_A\)Rs can be impaired when combined with other flavonoids that are commonly consumed or combined with DHM. With our combination studies using known concentrations of flavonoids that result in beneficial effects when used alone, we found that the addition of one of these several dietary ingredients/flavonoids when administered with DHM reduces DHM activity and the ability to counteract EtOH-mediated effects on GABA\(_A\)Rs (Figure 2).

As illustrated in Figure 2(A), DHM combined with 42 nM of turmeric, mink, resveratrol, or daidzein either significantly reduced the potentiation effects of DHM on GABA\(_A\)Rs (DHM + turmeric; *P < 0.001) or did not result in DHM potentiation as compared with DHM alone. On the other hand, DHM, in combination with citric acid (pH 3.6), resulted in similar effects to that of DHM alone (Figure 2(A)). To investigate these changes further, we tested the combination of ingredients on DHM’s ability to counteract EtOH-mediated GABA\(_A\)R activity (Figure 1(B)). As depicted in Figure 2(B), DHM combined with 42 nM of citric acid (pH 3.6), mink, and daidzein resulted in similar efficacy (*P < 0.001). However, the combination of DHM with 42 nM of turmeric and resveratrol reduced these effects (Figure 2(B)). Therefore, combining DHM with similar acting flavonoids appears to minimize the GABA\(_A\)R potentiation effects of DHM, along with its ability to counteract EtOH-mediated GABA\(_A\)R activity. These initial findings suggest that greater focus should be placed on the use of combinatorial herbal therapies to determine if the combined effects improve, reduce, or have no change in beneficial effects.

From our current findings, we find that the health benefits of DHM administration, as related to GABA\(_A\)R activity, can be severely reduced when mixed with other flavonoids. The combination of dietary supplements and health products that are available and/or marketed as containing DHM-like products is important to report because these combinations can reduce DHM-mediated benefits observed in preclinical animal and human studies.\(^3,6,12,13\) Furthermore, the evaluation of the safety of these compounds needs to be considered when combining them together, which may result in a reduction of DHM efficacy.

In conclusion, when considering the development of new food and dietary supplement products that contain DHM, one needs to consider the potentially deleterious effects of the added ingredients and overall efficacy of the product. Combining DHM with other health-promoting dietary ingredients may reduce the total benefits of DHM, and risk other...
adverse effects. Additional preclinical studies investigating the impact of these combinations are necessary and should be evaluated when considering the advancement of DHM to the market for its specific labeled benefits.

**Experimental**

**Reagents**

All flavonoids/herbs were purchased from Master Herbs Inc. (Pomona, CA, USA), and GABA from Sigma-Aldrich (St. Louis, MO, USA). Reagents were tested at 42 nM for combination studies or between 0.1 and 30 µM for GABA_A/R studies. EtOH concentrations for electrophysiology were tested at 60 mM.

**Electrophysiological Recordings**

Previously, we have found that DHM potentiates GABA_A/R activity and may play a role in antialcohol effects in rodent models.6 To identify other flavonoids with similar activity, electrophysiological recordings were conducted using DHM, daidzein, myricetin, resveratrol, and genistein between the concentrations of 0.1 µM and 30 µM. Similarly, studies were conducted combining DHM (42 nM) with 42 nM of turmeric, citric acid, mink, resveratrol, or daidzein to determine changes in DHM activity on GABA_A/Rs and counteracting EtOH effects on GABA_A/Rs. To conduct these studies, 4 male Sprague-Dawley rats (Harlan laboratory) were anesthetized with isoflurane, and their brains removed. Transverse slices (400 µm thick) of dorsal hippocampus were obtained using standard techniques.14,15 Whole-cell patch-clamp recordings were obtained from cells located in the CA1 pyramidal layer at 34 ± 0.5 °C during perfusion with artificial cerebrospinal fluid composed of 125 mM sodium chloride, 2.5 mM calcium chloride, 2 mM magnesium chloride, 26 mM sodium bicarbonate, and 10 mM d-glucose. The electrophysiology recordings, detection, and analysis flow of the dorsal hippocampus were conducted using the listed flavonoids and dietary supplements and following our previously described protocol.16,17 The investigator performing the recordings and mIPSC analysis was blind to experimental conditions.

**Loss of Right Reflex**

This assay is a well-established method for testing alcohol intoxication and tolerance and was performed as previously described.6,17 All animal protocols were performed according to the protocols approved by the University of Southern California (USC) Institutional Animal Care and Use Committee, and all methods were carried out in accordance with relevant guidelines and regulations. For this study, rats were administered a single dose of each supplement alone to determine whether the activity of the studied flavonoids and dietary supplements on GABA_A/Rs had any effect on EtOH intoxication (day 1) and withdrawal (day 3) using the LORR. In short, 8-10 rats/group received either 3 g/kg EtOH or 20 mL/kg saline (intraperitoneal [i.p.] injection) followed by drug gavage (10 mg/kg resveratrol, 20 mg/kg daidzein, 2 mg/kg myricetin, and 2 mg/kg DHM). After 2 days of withdrawal (day 3), all animals received another i.p. injection of EtOH 3 g/kg, including the saline control, to test alcohol withdrawal-induced tolerance. After EtOH injection and drug administration (gavage), a timer was started, and rats were placed in the supine position in a V-shaped support. LORR onset time was taken from the end-point of injection to the start of LORR. LORR ended when the animal was able to flip over 3 times in 30 seconds.

**Data Analysis**

Data are expressed as the mean ± SEM. Two-way analysis of variance, followed by multiple comparison analyses based on the Sidak method was performed. SigmaStat (Systat Software Inc. San Jose, CA, USA), SAS (V9.4, SAS Institute, Cary, NC, USA), and Graph-Pad Prism 6.0 were used. Differences among groups were stated to be statistically significant when P ≤ 0.05

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**References**

1. Bailey RL, Gahche JJ, Miller PE, Thomas PR, Dwyer JT. Why us adults use dietary supplements. JAMA Intern Med. 2013;173(5):355-361. doi:10.1001/jamainternmed.2013.2299
2. Wang F, Li Y, Zhang Y-J, Zhou Y, Li S, Li H-B. Natural products for the prevention and treatment of hangover and alcohol use disorder. Molecules. 2016;21(1):64-21. doi:10.3390/molecules21010064
3. Liang J, López-Valdés HE, Martínez-Coria H, Kerstin Lindemeyer A, Shen Y, et al. Dihydromyricetin ameliorates behavioral deficits and reverses neuropathology of transgenic mouse models
of Alzheimer's disease. Neurochem Res. 2014;39(6):1171-1181. doi: 10.1007/s11064-014-1304-4
4. Tomczyk M, Zovko-Koncić M, Chrostek L. Phytotherapy of alcoholism. Nat Prod Commun. 2012;7(2):273-280. doi:10.1177/1934578X1200700243
5. Szeto YT, Wong JWM, Wong SCY, Pak SC, Benzie IFF. DNA protective effect of ginseng and the antagonistic effect of Chinese turnip: a preliminary study. Plant Foods Hum Nutr. 2011;66(2):97-100. doi:10.1007/s11130-011-0209-5
6. Shen Y, Lindemeyer AK, Gonzalez C, et al. Dihydromyricetin as a novel anti-alcohol intoxication medication. J Neurosci. 2012;32(1):390-401. doi:10.1523/JNEUROSCI.4639-11.2012
7. Silva J, Yu X, Moradian R, et al. Dihydromyricetin protects the liver via changes in lipid metabolism and enhanced ethanol metabolism. Alcohol Clin Exp Res. 2020;44(5):1046-1060. doi:10.1111/acer.14326
8. Liang J, Shen Y, Shao XM, et al. Dihydromyricetin prevents fetal alcohol exposure-induced behavioral and physiological deficits: the roles of GABAA receptors in adolescence. Neurochem Res. 2014;39(6):1147-1161. doi:10.1007/s11064-014-1291-5
9. Li H, Li Q, Lü Z, et al. The versatile effects of dihydromyricetin in health. Evid Based Complement Alternat Med. 2017;2017(6):1-10. doi:10.1155/2017/1053617
10. Martínez-Coria H, Mendoza-Rojas MX, Arrieta-Cruz I, López-Valdés HE. Preclinical research of dihydromyricetin for brain aging and neurodegenerative diseases. Front Pharmacol. 2019;10(November):1-6. doi:10.3389/fphar.2019.01334
11. Ginwala R, Bhavsar R, Chigbu DI, Jain P, Khan ZK. Potential role of flavonoids in treating chronic inflammatory diseases with a special focus on the anti-inflammatory activity of apigenin. Antioxidants. 2019;8(2):35. doi:10.3390/antiox8020035
12. Pérez-Cano F, Flavonoids CM. Inflammation and immune system. Nutrients. 2016;8(10):639 doi:10.3390/nu8100659
13. Chen S, Zhao X, Wan J, et al. Dihydromyricetin improves glucose and lipid metabolism and exerts anti-inflammatory effects in nonalcoholic fatty liver disease: a randomized controlled trial. Pharmacol Res. 2015;99:74-81. doi:10.1016/j.phrs.2015.05.009
14. Spigelman I, Zhang L, Carlen PL. Patch-Clamp study of postnatal development of CA1 neurons in rat hippocampal slices: membrane excitability and K+ currents. J Neurophysiol. 1992;68(1):55-69. doi:10.1152/jn.1992.68.1.55
15. Kang M, Spigelman I, Sapp DW, Olsen RW. Persistent reduction of GABA(A) receptor-mediated inhibition in rat hippocampus after chronic intermittent ethanol treatment. Brain Res. 1996;709(2):221-228. doi:10.1016/0006-8993(95)01274-5
16. Liang J, Suryanarayanan A, Abriam A, Snyder B, Olsen RW, Spigelman I. Mechanisms of reversible GABAA receptor plasticity after ethanol intoxication. J Neurosci. 2007;27(45):12367-12377. doi:10.1523/JNEUROSCI.2786-07.2007
17. Liang J, Cagetii E, Olsen RW, Spigelman I. Altered pharmacology of synaptic and extrasynaptic GABAA receptors on CA1 hippocampal neurons is consistent with subunit changes in a model of alcohol withdrawal and dependence. J Pharmacol Exp Ther. 2004;310(3):1234-1245. doi:10.1124/jpet.104.067983