Research Article Open Access

A combination of multi-strain probiotics, prebiotic, and plant extracts improves ethanol-induced hangover outcomes in a zebrafish model

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Submission Date: September 10th, 2024; **Acceptance Date**: October 11th, 2024; **Publication Date:** October 17th, 2024

Please cite this article as: Ou Y., Zhang W., Lan J., Huang X., Li N. A combination of multi-strain probiotics, prebiotic, and plant extracts improves ethanol-induced hangover outcomes in a zebrafish model. *Functional Foods in Health and Disease* 2024; 14(10): 728-738. DOI: https://doi.org/10.31989/ffhd.v14i10.1462

ABSTRACT

Background: Alcohol use, even in moderation, causes hangover discomforts. It has been reported that alcohol-associated symptoms can be improved by either probiotics or medicinal plant extracts to some extent. However, the effects of a combination of multi-strain probiotics, prebiotic, and plant extracts have not been fully explored.

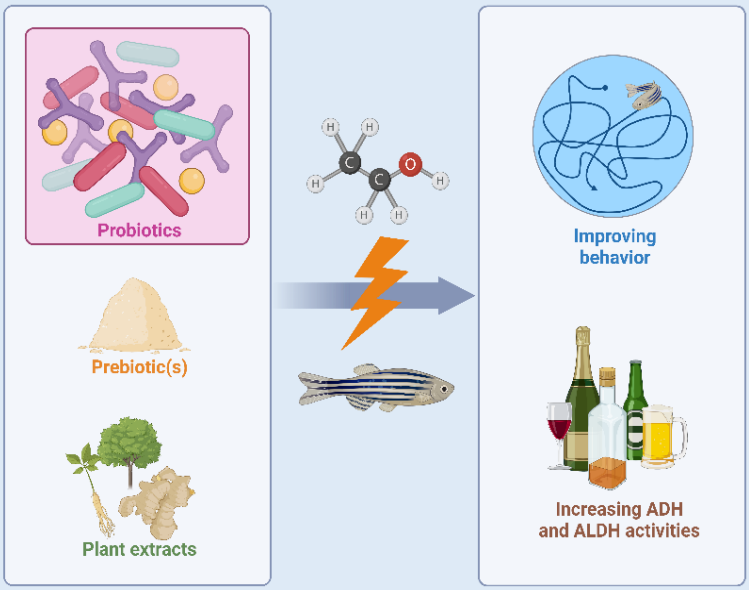
Objective: Our main goal is to assess behavioral responses and alcohol metabolism in zebrafish treated with a combination of multi-strain probiotics, prebiotic, and plant extracts after ethanol administration.

Methods: In this study, the zebrafish were first treated with the probiotic-prebiotic-plant extract mixture, and then was exposed to different levels of ethanol. The moving distance and activities of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) of zebrafish were measured after ethanol treatment.

Results: This probiotic-prebiotic-plant extract mixture significantly improved total distance moved and activities of ADH and ALDH in zebrafish treated with either 1.5% or 4.0% ethanol. In particular, the mixture outperformed plant extracts (both at 250μg/mL) in total distance moved under 1.5% ethanol administration and degrading acetaldehyde under 4.0% ethanol treatment.

Conclusion: A combination of probiotics, prebiotic, and plant extracts effectively mitigated hangover symptoms in cases of moderate and heavy alcohol use and demonstrated superior performance in acetaldehyde metabolism compared to plant extracts alone after heavy drinking, based on a zebrafish model.

Keywords: probiotic, prebiotic, Hovenia dulcis, kudzu root, curcumin, ethanol, hangover, behavior, metabolism, zebrafish.



Graphical Abstract: A combination of multi-strain probiotics, prebiotic, and plant extracts improves ethanol-induced hangover outcomes in a zebrafish model

INTRODUCTION

Alcohol consumption is a prevalent activity for social, cultural, recreational, stress-relief, and relaxation purposes. It varies largely between countries and regions. Europe exhibits the highest alcohol consumption with more than 12 liters of pure alcohol per person per year, while North Africa and the Middle East consume a notably lower amount of alcohol, ranging between 0 and 2 liters per person per year [1]. In China, each adult approximately consumes 6 to 8 litter pure alcohol annually [1]. Drinking can cause a series of uncomfortable physiological and psychological symptoms, including headache, nausea, diarrhea, fatigue, and shivering, along with reduced cognitive and visual abilities, and impaired ability to drive a vehicle or operate machinery; these symptoms are collectively termed as a hangover [2-3]. It is noteworthy that even moderate alcohol use can result in hangover symptoms.

Probiotics, as suggested by numerous studies, confer various health benefits to the host by regulating the gut microbiota, restoring intestinal barrier, reducing luminal endotoxin, and activating the immune response [4]. These functional characteristics may enable probiotics, such as *Lactobacillaceae* (including *Lacticaseibacillus* and *Lactobacillus*), *Bifidobacterium*, *Lactococcus*, *Saccharomyces*, to potentially address alcohol-relevant health issues. Nosova et al. found that *Lacticaseibacillus rhamnosus* exhibited excellent capability in degrading ethanol and acetaldehyde *in vitro* [5]. Recently, Vatsalya et al. found that a one-month intervention with *Lacticaseibacillus rhamnosus* GG significantly improved the severity of liver injury in patients with alcohol use disorder and alcohol-related hepatitis, though the study being a pilot one without

dose-response evaluations [6]. Furthermore, *Lacticaseibacillus casei*, another bacterium belonging to the family *Lactobacillaceae*, was reported to regulate lipid metabolism in both alcohol-treated rats and patients with alcohol liver injury [7-8]. Additionally, *Lactobacillus acidophilus* modulated colonic inflammation-related phenylalanine metabolism in mice with alcohol-associated liver injury [9]. *Bifidobacterium animalis* subsp. *lactis* KV9 directly reduced duration of loss of righting reflex and blood ethanol levels in alcohol-treated mice [10], while *Pediococcus pentosaceus* decreased inflammatory cytokines, improved intestinal barrier, and restored microbial communities in mice with ethanol-induced liver injury [11]. Moreover, Huang et al. observed quicker alcohol elimination in participants who consumed daily 10^{10} colony-forming units (CFU) of *Bacillus coagulans* TCI711 for one week, as indicated by breath alcohol tests [12]. Though being less studied, prebiotics, which are non-digestible carbohydrates (e.g., fructo-oligosaccharides and inulin), seem to enhance the ability of probiotics to alleviate hangover [13].

Apart from probiotics, several medicinal herbs (e.g., *Hovenia dulcis*, kudzu root, and curcumin) have long been recognized for their role in relieving hangover symptoms. For instance, *Hovenia dulcis* has been shown to reduce alcohol-induced liver injury in mice and improve hangover symptoms in human participants [14]. This relief may be attributed to dihydromyricetin, a flavonoid enriched in *Hovenia dulcis*, which regulates the BZ sites on $GABA_A$ receptors in the brain [15]. The anti-alcohol intoxication role of kudzu root is not only documented in classic texts of traditional Chinese medicine (i.e., Huang Di Nei Jing and Shang Han Lun) [16], but also supported by recent animal and clinical research [17-18]. Curcumin alleviates hangover discomfort by reducing oxidative stress and quickly removing reactive oxygen species [19].

To date, the effects of a combination of probiotics, prebiotics, and plant extracts on hangover improvement, particularly on behavioral changes, have not been explored in subjects without alcohol-associated disorders or diseases. Because of the high degree of genetic [20], neurological, and behavioral resemblance of zebrafish with human [21-22], we applied a zebrafish model to exploring ethanol-related problems of human. This study aims to investigate how such a mixture improves hangover symptoms in alcohol-treated zebrafish models, focusing on behavioral responses (i.e., total distance moved) and alcohol metabolism (i.e., activities of enzymes involved in ethanol and acetaldehyde degradation).

METHODS

Study design: A wild type zebrafish model of the AB strain (5 days postfertilization) was used in this study, of which the sex was not specified as sexual differentiation of zebrafish is initiated between 20 and 25 days postfertilization [23]. The zebrafish was housed in static environment, meeting the requirements of temperature at 28℃, conductivities ranging from 450 to 550 μS/cm, pH at 6.5 to 8.5, and water hardness between 50 to 100 mg/L $CaCO_3$.

A mixture of probiotics (including *Lacticaseibacillus rhamnosus*, *Lacticaseibacillus casei*, *Lactobacillus acidophilus*, *Bifidobacterium animalis*, and *Pediococcus pentosaceus*), prebiotic (i.e., fructooligosaccharide), and plant extracts (including ingredients derived from *Hovenia dulcis* containing 2% dihydromyricetin, kudzu root containing 5% puerarin, curcumin, and rice bran containing 78% long chain fatty alkanols) was evaluated in relieving ethanol-induced hangover outcomes in the zebrafish (ratio, the mixed probiotic powder: fructooligosaccharide: *Hovenia dulcis*: kudzu root: curcumin: rice bran = 2×10^{10} colony-forming units: 20mg: 75mg: 20mg: 5mg: 2.56mg). In addition, a plant-extract only group was included in this study for comparisons.

Since hangover outcomes induced by ethanol are dose-dependent (e.g., low levels of ethanol results in hyperactivity, while high levels of ethanol induce hypoactivity) [24–27], we included two ethanol doses (1.5% and 4.0% v/v) when exploring hangover-alleviating effects.

The study was approved by Institutional Animal Care and Use Committee (IACUC-2024-8587-01) and conducted according to the standards of Association for Assessment and Accreditation of Laboratory Animal Care (001458).

Determining maximum tolerated concentrations (MTCs): To explore the MTCs, we measured mortality rates in normal control (no intervention and no ethanol treatment), the probiotic-prebiotic-plant extract (PPP: with intervention of the mixture of probiotics, prebiotic, and plant extracts, as well as ethanol treatment), and the plant extract (PE: with intervention of plant extracts and ethanol administration) groups. Five concentrations were tested for both PPP and PE, including 125, 250, 500, 1000, and 2000μg/mL. Each group contained 30 zebrafish. The zebrafish were treated with different concentrations of PPP or PE for 24h, followed by ethanol treatment (1.5% or 4.0% v/v) for 1h. After that, the mortality rates were immediately measured.

Assessing total distance moved: In addition to normal control, ethanol control (no intervention but with ethanol treatment), PPP, and PE groups, we included a positive control group by using 100μg/mL RU-21. RU-21 is a commercial hangover-relief vitamin dietary supplement, widely used as a positive control in alcohol-related study [28–31]. The concentrations of PPP and PE were chosen based on MTC tests. The zebrafish (N=30 in each group) were first treated with RU-21, PPP, or PE, for 24h. Then, N=10 zebrafish were randomly selected and treated with ethanol (1.5% or 4.0% v/v) for 1h. During this period, total distance moved (path length, mm) was measured by zebrafish behavior system (Zebra Lab 3.22.3.31, Viewpoint, France).

Measuring enzyme activities of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH): When measuring ADH and ALDH, the most effective doses of PPP and PE were chosen in accordance with total distance moved results. The zebrafish (N=30 in each group; repeated for three times) were treated with RU-21, PPP, or PE, for 24h, followed by ethanol administration (1.5% or 4.0% v/v) for 1h. The zebrafish were collected in Eppendorf tubes added with 100μL saline solution (0.9% NaCl) and 5 to 8 glass beads (1mm). Samples were then homogenized in an automatic homogenizer (JXFSTPRP-24L, Jingxin, China) for 2 min at room temperature and 60Hz. Homogenized samples were centrifuged for 4 min at 4℃ and 12,000g. The supernatant of samples was kept and used for the determination of ADH and ALDH by ELISA kits and ELISA Analyzer (SPARK, TECAN, Austria).

Statistical analyses: Total distance moved after being normalized and levels of ADH and ALDH were analyzed and visualized by GraphPad Prism 8.0.2, with $p<0.05$ indicating significance.

RESULTS

MTCs in an ethanol-treated zebrafish model: To explore the MTCs, mortality rates were compared between groups (Table 1). Acceptable mortality rates (i.e., no more than 5%) were observed at the MTC of 250μg/mL of either PPP or PE in both 1.5% and 4.0% ethanol-treated zebrafish models.

Table 1. MTCs in 1.5% and 4.0% ethanol-treated zebrafish models.

Group	Concentration of PPP or PE (μg/mL)	Ethanol%	Mortality rates%	Chi-square *p* (vs normal control)
Normal control	0	0	0% (0/30)	-
PPP[1]	125	1.5	3% (1/30)	0.313
	250	1.5	3% (1/30)	0.313
	500	1.5	27% (8/30)	0.002
	1000	1.5	83% (25/30)	<0.001
	2000	1.5	100% (30/30)	<0.001
PE[2]	125	1.5	0% (0/30)	-
	250	1.5	0% (0/30)	-
	500	1.5	83% (25/30)	<0.001
	1000	1.5	100% (30/30)	<0.001
	2000	1.5	100% (30/30)	<0.001
Normal control	0	0	0% (0/30)	-
PPP	125	4.0	0% (0/30)	-
	250	4.0	0% (0/30)	-
	500	4.0	53% (16/30)	<0.001
	1000	4.0	100% (30/30)	<0.001
	2000	4.0	100% (30/30)	<0.001
PE	125	4.0	0% (0/30)	-
	250	4.0	0% (0/30)	-
	500	4.0	100% (30/30)	<0.001
	1000	4.0	100% (30/30)	<0.001
	2000	4.0	100% (30/30)	<0.001

[1]Probiotic-prebiotic-plant extracts.
[2]Plant extracts.

PPP and PE improved total distance moved of zebrafish with ethanol exposure: The treatments of 1.5% ethanol induced more motor activities as indicated by increased total distance moved of zebrafish, while 4.0% ethanol suppressed such movements as shown by reduced path length (Figure 1A and 1B).

In 1.5% ethanol models, all studied levels of PPP (i.e., 62.5, 125, and 250μg/mL) recovered the total distance moved to a normal level (Figure 1C). This improvement of total distance moved was comparative to the effectiveness of the positive control RU-21. As for PE, only groups at 125 and 250μg/mL significantly improved total distance moved. It is noteworthy that PPP outperformed PE at the concentration of 250μg/mL (Unpaired *t* test, p=0.038).

In 4.0% ethanol models, all measured levels of PPP alleviated total distance moved compared with ethanol control, but did not retrieve this behavioral outcome to a normal condition (Figure 1D). As for PE, it is like the results in 1.5% ethanol models that only 125 and 250μg/mL groups ameliorated total distance moved after ethanol treatment. No differences were observed between PPP and PE at the same concentration.

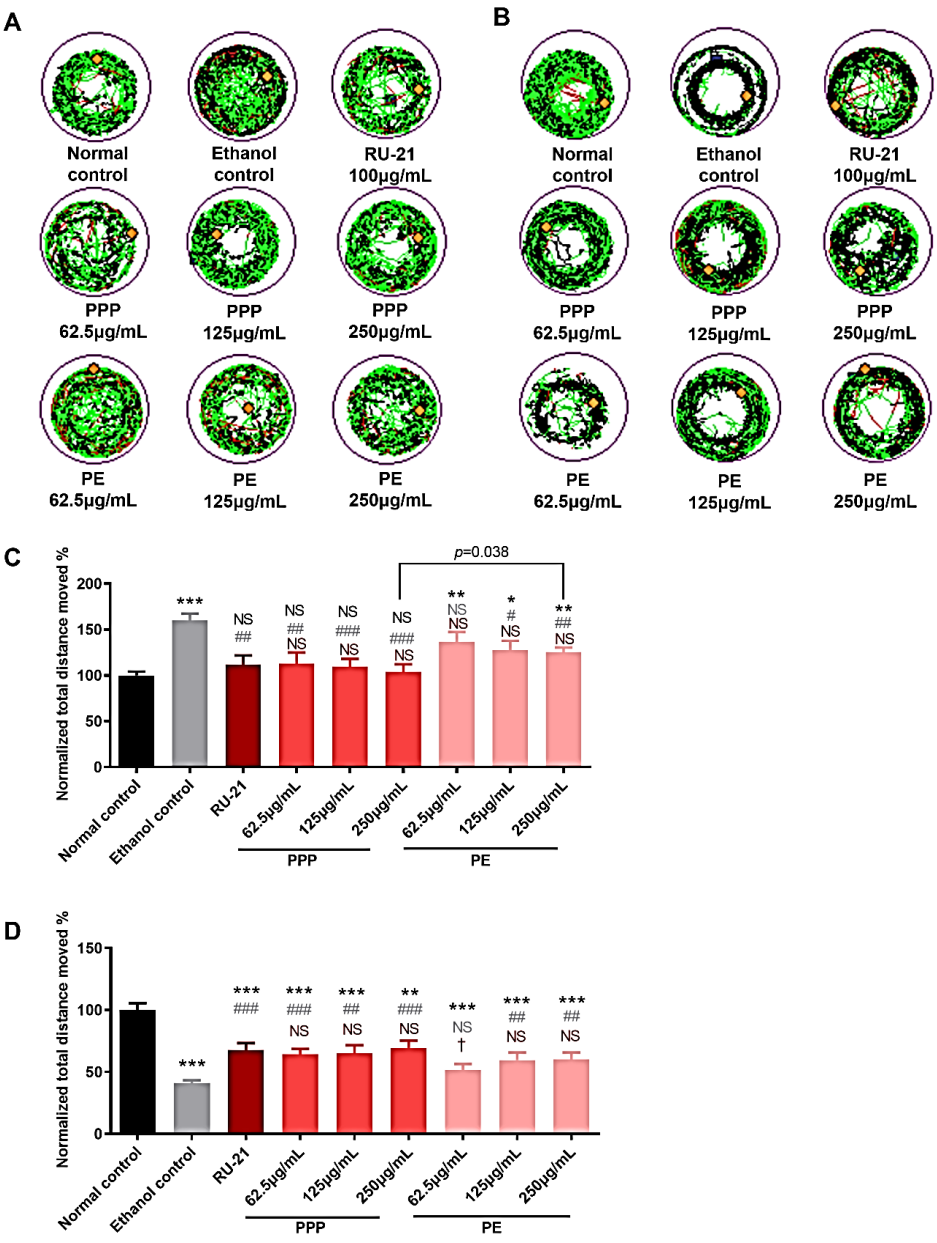


Figure 1. Total distance moved of zebrafish treated with ethanol. The moving trajectory of (A) 1.5% and (B) 4.0% ethanol-treated zebrafish models. Normalized path length of zebrafish administrated with (C) 1.5% and (D) 4.0% ethanol in reference to normal control. PPP, probiotic-prebiotic-plant extracts. PE, plant extracts. Differences were compared between groups by using Unpaired *t* tests when data follows normal distribution (or Nonparametric tests when data do not follow normal distribution). Comparisons of normal control with other groups are shown in black. Comparisons of ethanol control with RU-21, PPP, and PE groups are shown in grey. Comparisons of positive control RU-21 with PPP and PE groups are shown in brown. NS, not significant; *, #, †, $p < 0.05$; **, ##, $p < 0.01$; ***, ###, $p < 0.001$.

PPP and PE regulated ethanol metabolism by increasing ADH and ALDH activities: To depict ADH and ALDH activities with ethanol administration, the highest levels of PPP and PE (i.e., 250μg/mL) were used in zebrafish models. Compared with normal control, ethanol significantly reduced both ADH and ALDH activities in 1.5% and 4.0% ethanol models (Figure 2).

In 1.5% ethanol models, PPP restored ADH and ALDH activities to control group levels, while PE did not (Figure 2A and 2B). Moreover, PPP exhibited similar efficacy in improving ADH and ALDH to the positive control RU-21. Though no differences of ADH were observed between PPP and PE at the concentration of 250μg/mL, PPP contributed more improvements of ALDH

than PE, despite insignificant (Unpaired *t* test, *p*=0.066).

In parallel with the results in 1.5% ethanol models, PPP significantly improved ADH and ALDH activities to normal in 4.0% ethanol models (Figure 2C and 2D). Similar improvements of ALDH were also observed in RU-21 and PE but did not reach full restoration compared with normal control. Furthermore, PPP increased more ALDH activities than PE (Unpaired *t* test, *p*=0.018), along with a trend towards better performance in ADH recovery (Unpaired *t* test, *p*=0.065).

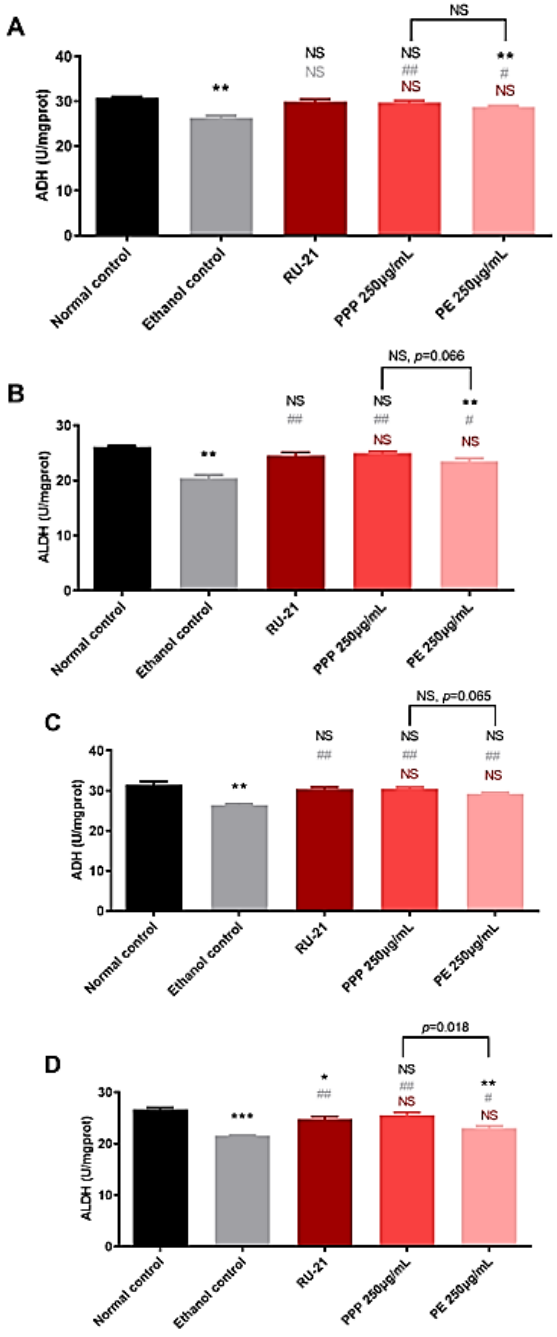


Figure 2. ADH and ALDH activities in ethanol-treated zebrafish models. (A) ADH and (B) ALDH under 1.5% ethanol treatment. (C) ADH and (D) ALDH under 4.0% ethanol administration. Differences were compared between groups by using Unpaired *t* tests when data follows normal distribution (or Nonparametric tests when data do not follow normal distribution). Comparisons of normal control with other groups are shown in black. Comparisons of ethanol control with RU-21, PPP, and PE groups are shown in grey. Comparisons of positive control RU-21 with PPP and PE groups are shown in brown. NS, not significant; *, #, $p < 0.05$; **, ##, $p < 0.01$; ***, $p < 0.001$.

DISCUSSION

The present study gained insight into the anti-hangover effects of a mixture of probiotics, prebiotic, and plant extracts in a model of zebrafish. In response to two different levels of ethanol (i.e., 1.5% and 4.0%), zebrafish exhibited excitation and inhibition on moving behavior, respectively. The probiotic-prebiotic-plant extract mixture improved the total distance moved by zebrafish in both ethanol models (comparative to the effectiveness of RU-21), directly indicating the mixture's hangover-alleviating effects in the cases of moderate and heavy

alcohol use. This efficacy may be attributed to increased activities of ADH and ALDH following the intervention. Importantly, the mixture demonstrated more profound improvements in total distance moved (1.5% ethanol models) and ALDH activity (4.0% ethanol models) compared to the plant extracts alone, suggesting potential positive interactions between probiotics, prebiotic, and plant extracts.

Consistent with previous research, our study using a mixture of probiotics, including *Lacticaseibacillus rhamnosus*, *Lacticaseibacillus casei*, *Lactobacillus acidophilus*, *Bifidobacterium animalis*, and *Pediococcus pentosaceus*, showed profound improvements on hangover in zebrafish exposed to ethanol. Among these five bacteria, the first three *Lactobacillaceae* genera were mostly reported in ameliorating alcohol-induced symptoms. *Lacticaseibacillus rhamnosus* demonstrated resistance to high levels of ethanol and production of enzymes that metabolize ethanol and acetaldehyde [5], with clinical evidence supporting its role in relieving liver injury in patients with alcohol-related disorders and diseases [6]. *Lacticaseibacillus casei* and *Lactobacillus acidophilus* improved alcohol-induced liver injury by regulating lipid metabolism and inflammatory conditions, respectively [7–9]. Additionally, *Bifidobacterium animalis*, another widely recognized probiotic, led to faster behavioral and cognitive recovery from alcohol-induced hangover in mice [10]. Although less investigated compared with *Lactobacillaceae* and *Bifidobacterium*, *Pediococcus pentosaceus* is attracting increasing attention as a promising probiotic, due to its antimicrobial abilities in producing bacteriocins, which can be extended to the functions of detoxification and anti-inflammation after alcohol use [11, 32].

It is noteworthy that the combination of probiotics and the plant extracts used in this study (i.e., *Hovenia dulcis*, kudzu root, and curcumin) boosted ALDH activity to a higher level than the plant extracts alone under 4.0% ethanol exposure. This may imply increasingly synergistic effects between the plant extracts and the probiotics at a high alcohol dose. The plant extracts contain several active ingredients, such as flavonoids, polyphenols, polysaccharides. These compounds, along with probiotics, can help maintain the integrity of the gut barrier under ethanol exposure by suppressing the growth of gastrointestinal pathogens [33]. Moreover, polyphenols are increasingly recognized as prebiotics, as certain probiotics possess the enzymatic capability to transform complex polyphenols into phenolic metabolites with increased bioavailability and antioxidative bioactivity [34]. During this procedure, polyphenols also aid the growth of beneficial microbes, thereby regulating the composition and function of the gut microbiota.

Importantly, in this study, we observed that the improving effects of the probiotic-prebiotic-plant extract mixture on behavioral response and ethanol metabolism of zebrafish were comparable to the effectiveness of a commercial hangover-relief vitamin dietary supplement RU-21, under both 1.5% and 4.0% ethanol treatments. This was like findings of previous studies comparing turmeric extracts [29], chicken hydrolysates [31], or traditional medicines with RU-21 [28]. Given that high doses of synthetic vitamin supplement may result in hypervitaminosis and intoxication [35], a combination of probiotic-prebiotic-plant extract is considered as a safer

approach in relieving hangover symptoms. Moreover, probiotics can adhere and colonize to the intestinal mucosa and therefore confer long-term benefits to host health through the gut-X axis [13, 36]. The benefits of taking probiotics in the format of fortified foods and dietary supplements mainly include supporting gut health, boosting immune system, and improving metabolic disorders [37–40]. In addition, as probiotics are alive microorganisms, suitable pH and low water and oxygen levels are required during processing and storage [41].

This present study was with some limitations: (1) Apart from ADH and ALDH activity measured in this study, in-depth analysis of the effects of the studied mixture on ethanol-related metabolism (e.g., levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and malonaldehyde, along with the activity of superoxide dismutase) needs to be conducted in future. (2) Though zebrafish was an ideal model in exploring alcohol-induced hangover symptoms, translational difficulties from animals to human must be taken into consideration. Therefore, the results of this study require further validations in exquisite-designed human studies. (3) The gut microbiota, which plays an intermediary role between dietary supplements (e.g., the combination of probiotic-prebiotic-plant extract mixture as mentioned in this study) and host health was not assessed in this study.

CONCLUSION

The supplementation of a mixture of probiotics, prebiotic, and plant extracts protected zebrafish exposed to moderate and heavy levels of ethanol from developing hangover symptoms. The anti-hangover effects of this mixture were ascribed to improved alcohol metabolism, as demonstrated by increased degradation of ethanol and acetaldehyde. These findings suggest that the combined use of probiotics, prebiotics, and plant extracts could be an effective strategy for mitigating the adverse effects of alcohol consumption.

Competing interests: The authors have no conflicts of interest to declare.

Authors' contributions: Y.O. and N.L. designed the study. Y.O. conducted the experiment and wrote the manuscript. J.L and X.H. prepared the samples. All authors revised the manuscript.

Abbreviations ADH: alcohol dehydrogenase, ALDH: aldehyde dehydrogenase, MTC: maximum tolerated concentrations, PPP: the probiotic-prebiotic-plant extract, PE: the plant extract

Acknowledgements: The author would like to thank the editors and the reviewers for their useful remarks.

Funding: This research did not receive any specific grant from funding agencies.

REFERENCES

1. Ritchie H, Roser M: Alcohol Consumption. 2022. [https://ourworldindata.org/alcohol-consumption], retrieved on October 17th, 2024
2. Devenney LE, Coyle KB, Verster JC: Memory and attention during an alcohol hangover. Human Psychopharmacology: Clinical and Experimental 2019. 34(4):e2701. DOI: https://doi.org/10.1002/hup.2701
3. Wiese JG, Shlipak MG, Browner WS: The Alcohol Hangover. 2000.
4. Mishra G, Singh P, Molla M, Yimer YS, Dinda SC, Chandra P, Singh BK, Dagnew SB, Assefa AN, Ewunetie A: Harnessing the potential of probiotics in the treatment of alcoholic liver disorders. Frontiers in Pharmacology 2023. 14:1212742. DOI: https://doi.org/10.3389/fphar.2023.1212742
5. Nosova T: Acetaldehyde production and metabolism by human indigenous and probiotic Lactobacillus and Bifidobacterium strains. Alcohol and Alcoholism 2000. 35(6):561–568. DOI: https://doi.org/10.1093/alcalc/35.6.561
6. Vatsalya V, Feng W, Kong M, Hu H, Szabo G, McCullough A, Dasarathy S, Nagy LE, Radaeva S, Barton B, Mitchell M, McClain CJ: The Beneficial Effects of Lactobacillus GG

Therapy on Liver and Drinking Assessments in Patients with Moderate Alcohol-Associated Hepatitis. The American Journal of Gastroenterology 2023. 118(8):1457–1460. DOI: https://doi.org/10.14309/ajg.0000000000002283

7. Li X, Han J, Liu Y, Liang H: *Lactobacillus casei* relieves liver injury by regulating immunity and suppression of the enterogenic endotoxin-induced inflammatory response in rats cotreated with alcohol and iron. Food Science and Nutrition 2021. 9(10):5391–5401. DOI: https://doi.org/10.1002/fsn3.2486
8. Li X, Liu Y, Guo X, Ma Y, Zhang H, Liang H: Effect of Lactobacillus casei on lipid metabolism and intestinal microflora in patients with alcoholic liver injury. European Journal of Clinical Nutrition 2021. 75(8):1227–1236. DOI: https://doi.org/10.1038/s41430-020-00852-8
9. Chen L, Yang P, Hu L, Yang L, Chu H, Hou X: Modulating phenylalanine metabolism by L. acidophilus alleviates alcohol-related liver disease through enhancing intestinal barrier function. Cell and Bioscience 2023. 13(1):24. DOI: https://doi.org/10.1186/s13578-023-00974-z
10. Zhang Z, Zhou H, Bai L, Lv Y, Yi H, Zhang L, Li R: Protective effects of probiotics on acute alcohol-induced liver injury in mice through alcohol metabolizing enzymes activation and hepatic TNF-α response reduction. Journal of Functional Foods 2019. 59:234–241. DOI: https://doi.org/10.1016/j.jff.2019.05.018
11. Jiang X-W, Li Y-T, Ye J-Z, Lv L-X, Yang L-Y, Bian X-Y, Wu W-R, et al.: New strain of *Pediococcus pentosaceus* alleviates ethanol-induced liver injury by modulating the gut microbiota and short-chain fatty acid metabolism. World Journal of Gastroenterology 2020. 26(40):6224–6240. DOI: https://doi.org/10.3748/wjg.v26.i40.6224
12. Huang C-H, Ho C-Y, Wang Y-L, Kan K-W, Chiu Y-K, Lin Y-H, Lin Y-H, Kuan C-M: The applications of B. coagulans TCI711 in alcohol elimination. International Journal of Food Sciences and Nutrition 2021. 72(5):713–719. DOI: https://doi.org/10.1080/09637486.2020.1858034
13. Sanders ME, Merenstein DJ, Reid G, Gibson GR, Rastall RA: Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. Nature Reviews Gastroenterology and Hepatology 2019. 16(10):605–616. DOI: https://doi.org/10.1038/s41575-019-0173-3
14. Kim H, Kim YJ, Jeong HY, Kim JY, Choi E-K, Chae SW, Kwon O: A standardized extract of the fruit of Hovenia dulcis alleviated alcohol-induced hangover in healthy subjects with heterozygous ALDH2: A randomized, controlled, crossover trial. Journal of Ethnopharmacology 2017. 209:167–174. DOI: https://doi.org/10.1016/j.jep.2017.07.028
15. Shen Y, Lindemeyer AK, Gonzalez C, Shao XM, Spigelman I, Olsen RW, Liang J: A single dose of kudzu extract reduces alcohol consumption in a binge drinking paradigm. The Journal of Neuroscience 2012. 32(1):390–401. DOI: https://doi.org/10.1523/JNEUROSCI.4639-11.2012
16. Keung WM, Vallee BL: Kudzu root: An ancient chinese source of modern antidipsotropic agents. Phytochemistry 1998. 47(4):499–506. DOI: https://doi.org/10.1016/S0031-9422(97)00723-1
17. Lukas SE, Penetar D, Berko J, Vicens L, Palmer C, Mallya G, Macklin EA, Lee DY-W: An Extract of the Chinese Herbal Root Kudzu Reduces Alcohol Drinking by Heavy Drinkers in a Naturalistic Setting: Alcoholism: Clinical and Experimental Research 2005. 29(5):756–762. DOI: https://doi.org/10.1097/01.ALC.0000163499.64347.92
18. Penetar DM, Toto LH, Lee DY-W, Lukas SE: A single dose of kudzu extract reduces alcohol consumption in a binge drinking paradigm. Drug and Alcohol Dependence 2015. 153:194–200. DOI: https://doi.org/10.1016/j.drugalcdep.2015.05.025
19. Farzaei M, Zobeiri M, Parvizi F, El-Senduny F, Marmouzi I, Coy-Barrera E, Naseri R, Nabavi S, Rahimi R, Abdollahi M: Curcumin in Liver Diseases: A Systematic Review of the Cellular Mechanisms of Oxidative Stress and Clinical Perspective. Nutrients 2018. 10(7):855. DOI: https://doi.org/10.3390/nu10070855
20. Howe K, Clark MD, Torroja CF, Torrance J, Berthelot C, Muffato M, Collins JE, et al.: The zebrafish reference genome sequence and its relationship to the human genome. Nature 2013. 496(7446):498–503. DOI: https://doi.org/10.1038/nature12111
21. Gerlai R, Lahav M, Guo S, Rosenthal A: Drinks like a fish: zebra fish (Danio rerio) as a behavior genetic model to study alcohol effects. Pharmacology Biochemistry and Behavior 2000. 67(4):773–782. DOI: https://doi.org/10.1016/S0091-3057(00)00422-6
22. Saleem S, Kannan RR: Zebrafish: an emerging real-time model system to study Alzheimer's disease and neurospecific drug discovery. Cell Death Discovery 2018. 4(1):45. DOI: https://doi.org/10.1038/s41420-018-0109-7
23. Kossack ME, Draper BW: Genetic regulation of sex determination and maintenance in zebrafish (Danio rerio). Current Topics in Developmental Biology 2019. 134:119–149. DOI: https://doi.org/10.1016/bs.ctdb.2019.02.004
24. De Esch C, Van Der Linde H, Slieker R, Willemsen R, Wolterbeek A, Woutersen R, De Groot D: Locomotor activity

assay in zebrafish larvae: Influence of age, strain and ethanol. Neurotoxicology and Teratology 2012. 34(4):425–433. DOI: https://doi.org/10.1016/j.ntt.2012.03.002

25. Irons TD, MacPhail RC, Hunter DL, Padilla S: Acute neuroactive drug exposures alter locomotor activity in larval zebrafish. Neurotoxicology and Teratology 2010. 32(1):84–90. DOI: https://doi.org/10.1016/j.ntt.2009.04.066
26. Karlsson O, Roman E: Dose-dependent effects of alcohol administration on behavioral profiles in the MCSF test. Alcohol 2016. 50:51–56. DOI: https://doi.org/10.1016/j.alcohol.2015.10.003
27. Lockwood B, Bjerke S, Kobayashi K, Guo S: Acute effects of alcohol on larval zebrafish: a genetic system for large-scale screening. Pharmacology Biochemistry and Behavior 2004. 77(3):647–654. DOI: https://doi.org/10.1016/j.pbb.2004.01.003
28. Chen C, Wen D-C, Gao S, Hu X, Yi C: The Protective Effects of Buzui on Acute Alcoholism in Mice. Evidence-Based Complementary and Alternative Medicine 2016. 2016:1–8. DOI: https://doi.org/10.1155/2016/3539748
29. Liang X, Wang Y, Hu X, Wang S: The Protective Effects of Water Extracts of Compound Turmeric Recipe on Acute Alcoholism: An Experimental Research Using a Mouse Model. Evidence-Based Complementary and Alternative Medicine 2021. 2021:1–13. DOI: https://doi.org/10.1155/2021/6641919
30. Shi P, Chen B, Chen C, Xu J, Shen Z, Miao X, Yao H: Honey reduces blood alcohol concentration but not affects the level of serum MDA and GSH-Px activity in intoxicated male mice models. BMC Complementary and Alternative Medicine 2015. 15(1):225. DOI: https://doi.org/10.1186/s12906-015-0766-5
31. Xiao C, Zhou F, Zhao M, Su G, Sun B: Chicken breast muscle hydrolysates ameliorate acute alcohol-induced liver injury in mice through alcohol dehydrogenase (ADH) activation and oxidative stress reduction. Food and Function 2018. 9(2):774–784. DOI: https://doi.org/10.1039/C7FO01387F
32. Jiang S, Cai L, Lv L, Li L: Pediococcus pentosaceus, a future additive or probiotic candidate. Microbial Cell Factories 2021. 20(1):45. DOI: https://doi.org/10.1186/s12934-021-01537-y
33. Li Z, Fang X, Hu X, Li C, Wan Y, Yu D: Amelioration of alcohol-induced acute liver injury in C57BL/6 mice by a mixture of TCM phytochemicals and probiotics with antioxidative and anti-inflammatory effects. Frontiers in Nutrition 2023. 10:1144589. DOI: https://doi.org/10.3389/fnut.2023.1144589
34. Rodríguez-Daza MC, Pulido-Mateos EC, Lupien-Meilleur J, Guyonnet D, Desjardins Y, Roy D: Polyphenol-Mediated Gut Microbiota Modulation: Toward Prebiotics and Further. Frontiers in Nutrition 2021. 8:689456. DOI: https://doi.org/10.3389/fnut.2021.689456
35. Rutkowski M, Grzegorczyk K: Adverse effects of antioxidative vitamins. International Journal of Occupational Medicine and Environmental Health 2012. 25(2):105–21. DOI: https://doi.org/10.2478/S13382-012-0022-x
36. Lau LYJ, Quek SY: Probiotics: Health benefits, food application, and colonization in the human gastrointestinal tract. Food Bioengineering 2024. 3(1):41–64. DOI: https://doi.org/10.1002/fbe2.12078
37. Patrick M, Peter C, Mary M: The potential health benefits of a novel synbiotic yogurt fortified with purple-leaf tea in modulation of gut microbiota. Bioactive Compounds in Health and Disease 2024. DOI: https://doi.org/10.31989/bchd.v7i4.1323
38. Joller P, Cabaset S, Maurer S: Influence of a Food Supplement on the Gut Microbiome in Healthy Overweight Women. Functional Foods in Health and Disease 2020. 10(10):428. DOI: https://doi.org/10.31989/ffhd.v10i10.750
39. Peng Y, Teng X, Liu T, Li Y, Ni J, Xue S, Wang J: Effect of an oral probiotic formula on scalp and facial skin condition, glucose, and lipid metabolism. Functional Foods in Health and Disease 2022. 12(7):394. DOI: https://doi.org/10.31989/ffhd.v12i7.944
40. Rithi AT, Mitra A, Banerjee A, Ilanchoorian D, Marotta F, Radhakrishnan AK: Effect of prebiotics, probiotics, and synbiotics on gut microbiome in diabetes among coastal communities. Functional Food Science 2024. 4(1):11. DOI: https://doi.org/10.31989/ffs.v4i1.1271
41. Tripathi MK, Giri SK: Probiotic functional foods: Survival of probiotics during processing and storage. Journal of Functional Foods 2014. 9:225–241. DOI: https://doi.org/10.1016/j.jff.2014.04.030