Research Article Open Access

Regulation of a synbiotic herbal-probiotic formula (HBF) on alcohol metabolism in human: A randomized controlled trial

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Submission date: September 11th, 2025; **Acceptance Date:** September 25th, 2025, **Publication Date:** October 1st, 2025

Please cite this article as: Sun C., Li F., Zhao H., Zhang P., Zhang Y., Jiang L., Zhao T., Chen L., Li Q., Lin D. Regulation of a synbiotic herbal-probiotic formula (HBF) on alcohol metabolism in human: A randomized controlled trial. *Functional Foods in Health and Disease*. 2025; 15(10): 688 – 700. DOI: https://doi.org/10.31989/ffhd.v15i10.1761

ABSTRACT

Background: Excessive alcohol intake is a global health concern. Short-term alcohol consumption can lead to unpleasant hangover experiences, while long-term excessive intake is associated with liver injury, neurological disorders, cancer, and various other diseases. Herbal and probiotic interventions have shown promise in modulating alcohol metabolism, but the effects of combined synbiotic formulas remain underexplored.

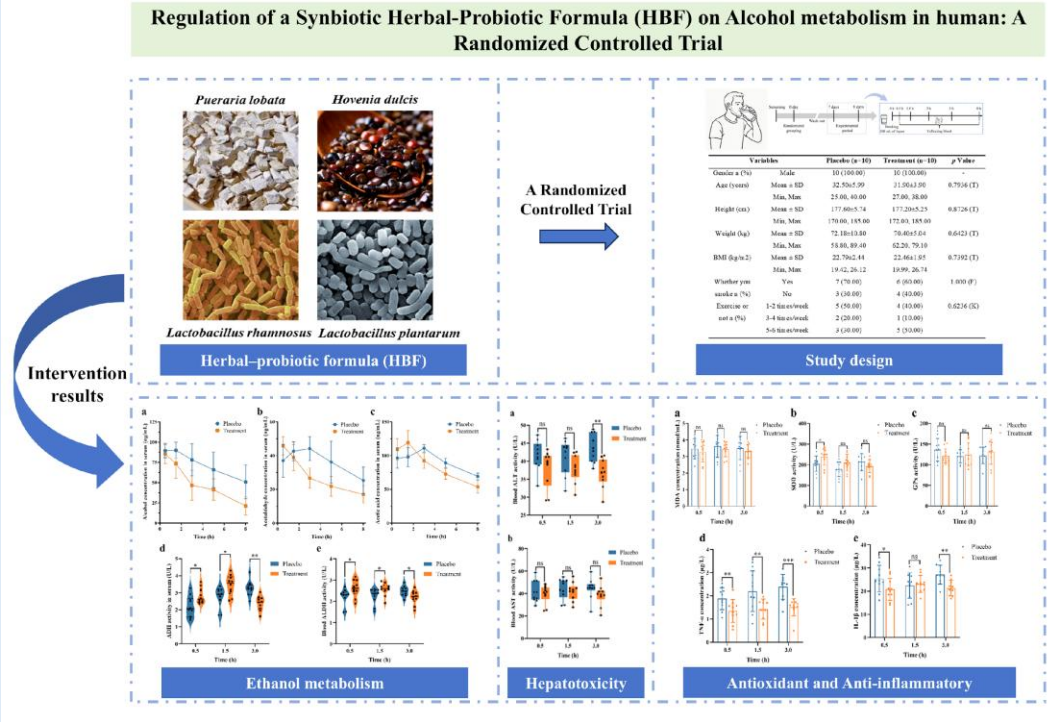
Methods: This study aimed to investigate the effects of a novel herbal–probiotic formula (HBF), composed of *Pueraria lobata* (30%), *Hovenia dulcis* (30%), *Lactobacillus rhamnosus* Alcoker0067 (20%), and *Lactobacillus plantarum* ZQ15 (20%), on alcohol metabolism in healthy male volunteers. In a randomized, placebo-controlled trial, 20 participants were assigned to either the HBF or placebo group. Following HBF or placebo administration and subsequent alcohol consumption, blood samples were collected at multiple time points (0.5–8 h) to measure ethanol, acetaldehyde, and acetic acid concentrations, as well as ADH and ALDH activities. Liver function (ALT, AST), oxidative stress markers (MDA, SOD, GPx), and inflammatory factors (TNF-α, IL-1β) were also evaluated.

Results: HBF significantly ($p < 0.05$) accelerated ethanol clearance and enhanced serum ADH and ALDH activities at 0.5 and 1.5 h. Compared to the placebo group, HBF administration led to lower serum ethanol and MDA levels, elevated

SOD activity, and reduced TNF-α and IL-1β concentrations, especially at 0.5 h and 3 h. ALT and AST levels were also decreased, indicating a hepatoprotective effect.

Conclusion: The HBF formula effectively promotes alcohol metabolism and alleviates oxidative stress and inflammation associated with alcohol consumption, supporting its potential as a safe and effective anti-hangover intervention.

Keywords: anti-hangover, alcohol, *Pueraria lobata*, *Hovenia dulcis*, *Lactobacillus rhamnosus* Alcoker0067, *Lactobacillus plantarum* ZQ15.



Graphical Abstract: Regulation of a synbiotic herbal-probiotic formula (HBF) on alcohol metabolism in human

INTRODUCTION

Alcohol is the most widely used psychoactive substance in the world, alcohol per-capita consumption is projected to increase further to 7.6 L in 2030 [1]. Chinese alcohol culture has a long and profound history, with a large amount of alcohol consumed annually. Between 1990 and 2017, the annual per capita alcohol consumption rose from 7.1–11.2 L, and it is expected to continue increasing in the coming years among Chinese men [2]. Hangover refers to the psychological and physiological effects that occur after drinking alcohol. It typically manifests as a range of discomforts, including but not limited to tiredness, headache, nausea, drowsiness, and vomiting [3]. Meanwhile, excessive alcohol consumption can lead to a series of diseases, such as alcoholic liver diseases [4], hypertension [5], heart attack [6], cancer [7] et al.

Alcohol is primarily absorbed through the stomach and small intestine in humans. The liver, as the main organ, can metabolize approximately 90% of the ingested alcohol. Ingested alcohol is first oxidized to be acetaldehyde by alcohol dehydrogenase (ADH), which is

then further oxidized by aldehyde dehydrogenase (ALDH) into the non-toxic acetic acid [8]. The intermediate metabolite, acetaldehyde, can exacerbate the symptoms of alcohol intoxication. The cytosolic ALDH1 and mitochondrial ALDH2 are two main isoforms of ALDH. However, approximately 30–50% of East Asians have ALDH2 deficiency due to the presence of a genetic variant known as the ALDH2*2 allele [9]. In fact, the rapid degradation of alcohol can alleviate the severity of hangovers and help maintain overall health [10]. Therefore, treatments that result in the early metabolism of alcohol are an effective strategy for preventing the harmful effects of alcohol abuse.

Many traditional Chinese herbal medicines have the ability to modulate alcohol metabolism, is attributable to their bioactive compounds. Bioactive compounds are key components in the development of functional foods that offer health benefits beyond basic nutrition [11]. *Pueraria lobata* and *hovenia dulcis* are widely used in clinical practice and the health food industry for their detoxifying effects against alcohol [12, 13]. As a drug pair, these two herbs form a fixed combination that enhances efficacy and has demonstrated protective effects against ethanol-induced liver injury in vitro [13]. Probiotics are beneficial microorganisms capable of modulating the gastrointestinal tract to favor an anti-inflammatory milieu, thereby attenuating pathogenic toxin production and strengthening the integrity of the gut barrier [14]. Recently, a randomized, double-blind, placebo-controlled crossover clinical trial demonstrated that a probiotic mixture of *Lactobacillus* and *Bifidobacterium* effectively reduced concentrations of alcohol and acetaldehyde [15]. However, few studies have focused on the effects of combining traditional Chinese herbal medicine with probiotics on alcohol metabolism.

According to the Functional Food Center (FFC), functional foods are defined as natural or processed foods containing bioactive compounds that, in effective and safe amounts, provide clinically proven health benefits beyond basic nutrition. Following this framework, the present study proposes a new herbal-probiotic formula (HBF) as a potential functional food, composed of *Pueraria lobata*, *Hovenia dulcis,* *Lactobacillus rhamnosus* Alcoker0067, and *Lactobacillus plantarum* ZQ15. The current study investigated the effects of HBF on blood alcohol, acetaldehyde, and acetic acid concentration. And the ADH and ALDH level were measured for elucidating a potential anti-hangover mechanism. In addition, the levels of inflammatory factors and oxidative stress markers in human blood were detected to comprehensively assess the efficacy of HBF on alcohol hangovers.

MATERIALS AND METHODS

Herbal-probiotic formula preparation: The HBF consists of *Pueraria lobata* (30%), *Hovenia dulcis* (30%), *Lactobacillus* rhamnosus Alcoker0067 (20%), and *Lactobacillus plantarum* ZQ15 (20%). The *Pueraria lobata* or Hovenia dulcis was obtained according to previous method [16]. 50 g of Pueraria lobata or Hovenia dulcis was added to 1 L of distilled water and soaked for 2 hours, followed by decoction at boiling temperature for another 2 hours. This extraction process was repeated twice. The extracts were filtered and combined, concentrated using rotary evaporation, and subsequently freeze-dried. The probiotic dose was 9.7×10^8 CFU/g HBF in the study.

Determination of total flavonoid and polyphenol: The total flavonoid content was determined using the aluminum trichloride colorimetric method, with rutin (SR8250, Solaibao, Beijing, China) as the standard [17]. The total polyphenol was measured using the Folin–Ciocalteu method with gallic acid (SG8040, Solaibao, Beijing, China) as the standard [18]. Each sample was tested in triplicate.

Recruitment and screening of participants: Participants were required to meet the following inclusion criteria: (1) Healthy male adults aged 18 to 50 years; (2) No known

allergy or intolerance to alcohol; (3) No history of severe or unstable physical disorders involving the cardiovascular, hepatic, renal, endocrine, digestive, or hematologic systems; (4) Provided written informed consent and demonstrated willingness to take the investigational product. The current study did not include female, as the menstrual cycle may affect alcohol intake behaviors and metabolic responses, potentially confounding the results [19].

Study design: The randomized controlled trial was approved by the Ethics Committee of China Agricultural University (ethics number CAUHR−20240902). The registration number of the Clinical Research Information Service is ChiCTR2400092361. All procedures were conducted in accordance with relevant guidelines and regulations. The overall intervention study procedure was illustrated in Figure 1. All participants refrained from consuming any alcoholic products during the week preceding the formal experiment. The subjects were randomly divided into two groups (placebo group and treatment group). The treatment group received 5 g HBF with 150 mL water. The placebo group only consumed 150 mL water. After 30 min, the volunteers ingested 100 mL Fenjiu (43% alcohol), which is a commercially available liquor. Following alcohol intake, venous blood samples were drawn by a trained nurse at 0.5, 1.5, 3, 5, and 8 h post-consumption.

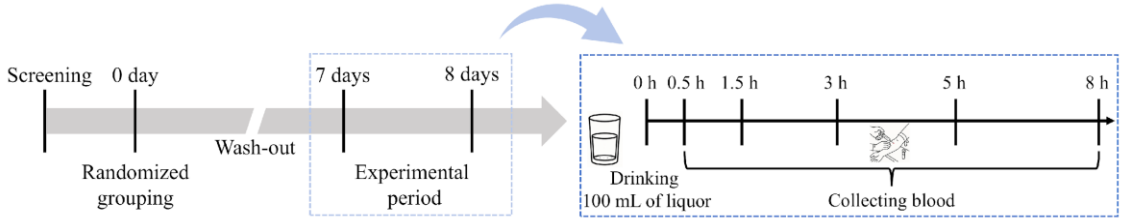


Figure 1. The flowchart of the clinical intervention trial after alcohol consumption.

Efficacy assessment: The blood sample was centrifuged at 4000 rpm for 15 minutes at 4 ℃ to obtain serum. At 0.5, 1.5, 3, 5, 8 h, the levels of ethanol, acetaldehyde, and acetic acid were measured. Commercial assay kits for ethanol (YX-052008H; Sino Best Biological Technology Co., Ltd., Shanghai, China), acetaldehyde (YX-010305H; Sino Best Biological Technology Co., Ltd., Shanghai, China) and acetic acid (YX-010100H; Sino Best Biological Technology Co., Ltd., Shanghai, China) were used according to the manufacturer's instructions. ADH and ALDH, as the markers of alcohol metabolism, were analyzed in serum samples using commercial kits for ADH (YX-010408H; Sino Best Biological Technology Co., Ltd., Shanghai, China) and ALDH (YX-010420H; Sino Best Biological Technology Co., Ltd., Shanghai, China).

Safety assessments: The blood levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured using commercial assay kits. Oxidative stress markers, including malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPX), as well as pro-inflammatory cytokines, tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β), were quantified using commercial kits. All assay kits used in the above analyses were provided by Sino Best Biological Technology Co., Ltd. (Shanghai, China).

Statistical analysis: Statistical analyses were performed using IBM SPSS Statistics software (version 22.0; Chicago, USA). The results from this clinical trial were expressed as the means ± standard deviation. $p < 0.05$ was considered to have significant differences. An independent-samples

t-test was used for comparisons within two independent sets of data. Graph plotting was performed using GraphPad Prism 10.1.2, Origin 2022.

RESULTS

Enrollment: The use of well-defined enrollment criteria and detailed participant flow is critical for maintaining sample integrity, thereby enhancing the reliability of the study and ensuring accurate interpretation of the findings. Figure 2 outlines the comprehensive recruitment pathway and screening standards applied to all enrolled participants. This study enrolled 29 participants, following 5 individuals excluded during the screening process, which included alcohol intolerance, hematophobia, and hypertension. Subsequently, the 24 volunteers were randomly divided into two groups. Four participants were excluded due to vomiting and insufficient blood sample during the trial period. Additionally, written informed consent was obtained from all participants before the initiation of the study. Ultimately, 10 participants in both the placebo and treatment groups completed the trial.

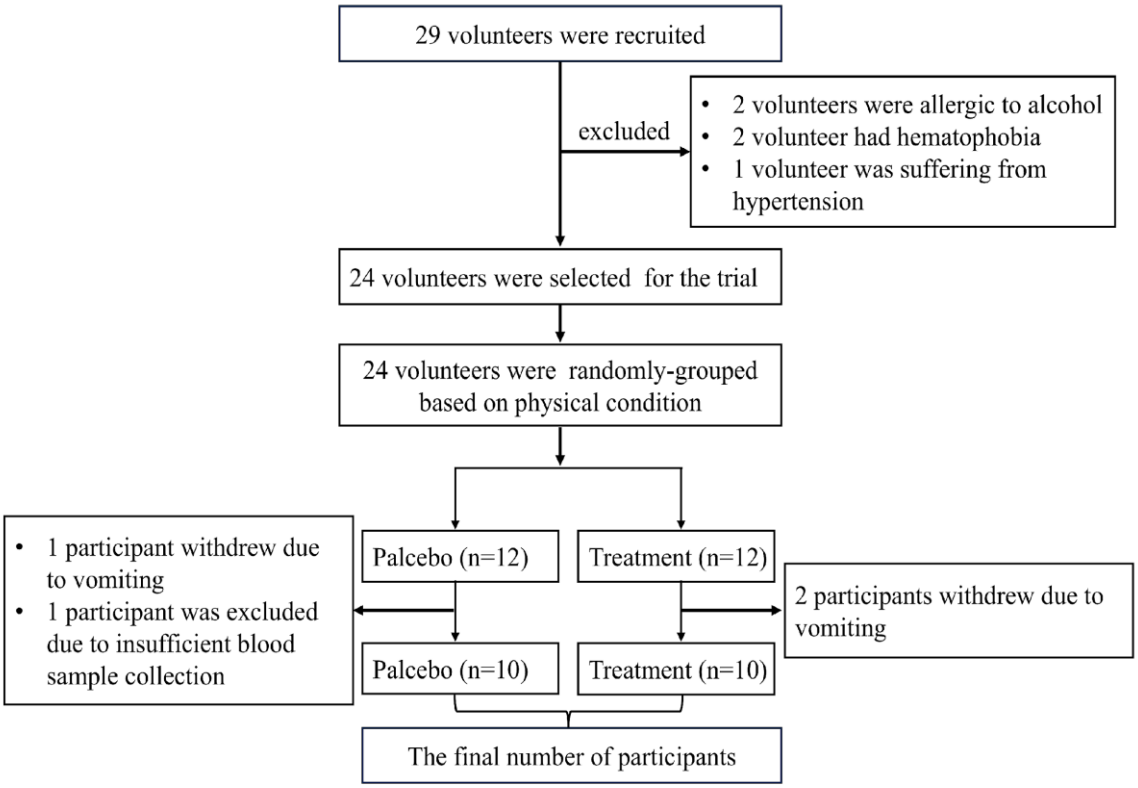


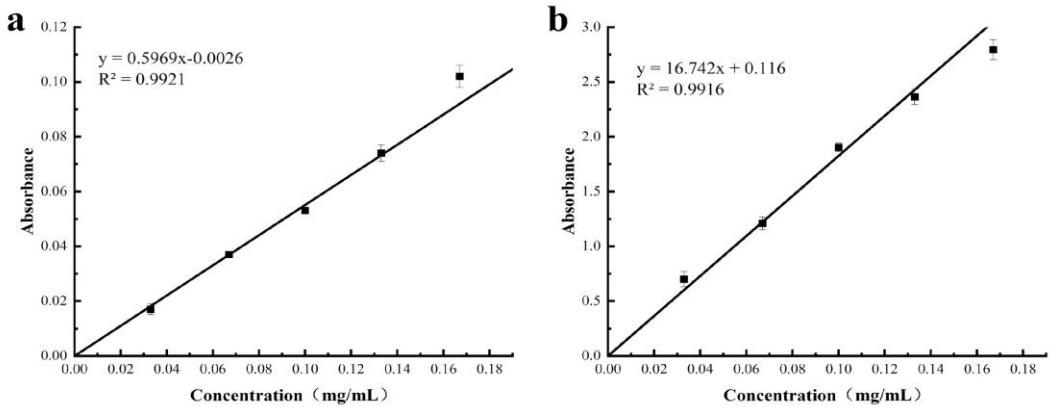
Figure 2. Recruitment and screening of study participants.

General Participant Characteristics: Table 1 provides a comparison of the demographic characteristics of the final participants. All characteristics, including demographic variables, were thoroughly assessed between Placebo and Treatment to determine the presence of any statistically significant differences. All participants were male in the study. The analysis showed that there were no statistically significant differences between the groups regarding age, height, weight, BMI, smoking and exercise. The results suggest that randomization was effectively achieved, which strengthens both the reliability and validity of the study outcomes. Furthermore, the findings suggest that these demographic variables did not act as confounders in the context of this study.

Table 1. Summary of demographic information and pre-ingestion characteristics of the subjects. *p*-value for or Fisher's exact test (F) in categorical variables, p-value for independent-samples *t* test (T) or Kruskal–Wallis test (K) in continuous variables.

Variables		Placebo (n=10)	Treatment (n=10)	*p* Value
Gender n (%)	Male	10 (100.00)	10 (100.00)	-
Age (years)	Mean ± SD	32.50±5.99	31.90±3.90	0.7936 (T)
	Min, Max	25.00, 40.00	27.00, 38.00	
Height (cm)	Mean ± SD	177.60±5.74	177.20±5.25	0.8726 (T)
	Min, Max	170.00, 185.00	172.00, 185.00	
Weight (kg)	Mean ± SD	72.18±10.80	70.40±5.04	0.6423 (T)
	Min, Max	58.80, 89.40	62.20, 79.10	
BMI (kg/m2)	Mean ± SD	22.79±2.44	22.46±1.95	0.7392 (T)
	Min, Max	19.42, 26.12	19.99, 26.74	
Whether you smoke n (%)	Yes	7 (70.00)	6 (60.00)	1.000 (F)
	No	3 (30.00)	4 (40.00)	
Exercise or not n (%)	1-2 times/week	5 (50.00)	4 (40.00)	0.6236 (K)
	3-4 times/week	2 (20.00)	1 (10.00)	
	5-6 times/week	3 (30.00)	5 (50.00)	

The Polyphenol and Flavonoid Content in Pueraria Lobata or Hovenia Dulcis Extracts: Polyphenols and flavonoids are the main functional active compounds in *Pueraria lobata* and *Hovenia dulcis*, which play a protective role in antioxidant and liver protective effect [20]. Therefore, the flavonoid and polyphenol contents in the *Pueraria lobata* and Hovenia dulcis extracts were determined, as shown in Figure 3. In fact, flavonoids, as an important branch, belong to polyphenols. Figure 3 a and Figure 3 b present the standard curves used for the quantification of polyphenols and flavonoids, respectively. As can be seen from Figure 3 c, each gram of HBF contains 124.86 mg of polyphenols and 90.12 mg of flavonoids.



c

	Flavonoid (mg/g)	Polyphenol (mg/g)
Pueraria lobata	102.4 ± 3.7	136.5 ± 4.2
Hovenia dulcis	47.8 ± 2.1	71.6 ± 3.4

Figure 3. Determination of total polyphenol and flavonoid contents. (a) The quantification of flavonoid based on a standard curve constructed with rutin as standard. (b) The quantification of polyphenol based on a standard curve constructed with garlic acid as standard. (c) The content of total polyphenol and flavonoid in *pueraria lobata* or *hovenia dulcis* extracts freeze-dried powder.

Regulation of Ethanol Metabolism by HBF: Figure 4 a–c show the changes in blood alcohol, acetaldehyde and acetic acid concentrations within 8 hours after ingestion alcohol. As shown in Figure 4 a, blood ethanol concentrations decreased over time in both the treatment and control groups. However, the treatment group exhibited a significantly faster decline. Notably, the average ethanol levels in the treatment group were consistently lower at all time points, with the most pronounced difference observed at 8 hours post-ingestion (21.19 ng/mL vs. 50.90 ng/mL). As shown in Figure 4 b, the blood acetaldehyde concentration in the treatment group peaked earlier and then declined more rapidly than in the control group. Although the treatment group exhibited a higher acetaldehyde level at 0.5 h (45.81 ng/mL vs. 36.98 ng/mL), it maintained consistently lower concentrations from 1.5 h onwards, with a maximum difference observed at 3 h (26.71 ng/mL vs. 44.15 ng/mL). These results suggest that HBF may accelerate acetaldehyde clearance following ethanol ingestion. Blood acetic acid levels increased rapidly in the treatment of HBF group during the early phase (0.5–1.5 h) and then declined more quickly compared to the control group. The peak acetate concentration in the treated group was observed at 1.5 h (119.17 ng/mL), significantly higher than the control group (97.83 ng/mL). However, from 3 h onward, the treatment group maintained consistently lower levels, indicating a potentially enhanced metabolic clearance of acetate facilitated by HBF.

It has been reported that blood alcohol levels generally reach their peak around one hour after alcohol drinking, followed by a progressive decrease [21]. Therefore, we measured the activities of ADH and ALDH in blood at 0.5, 1.5, 3 h (Figure 4 d, e). As shown in Figure 4 d, ADH activity was significantly ($p < 0.05$) higher in the treatment group at both 0.5 h (2.81 U/L vs. 2.19 U/L) and 1.5 h (3.45 U/L vs. 2.75 U/L). However, ADH activity in the treatment group declined below that of the control group at 3 h. In Figure 4 e, ALDH activity was also significantly higher ($p < 0.05$) in the treatment group at 0.5 h (2.61 U/L vs. 2.21 U/L) and 1.5 h (2.57 U/L vs. 2.24 U/L). However, the placebo group exhibited higher ALDH activity (2.48 U/L vs. 2.19 U/L) at 3h.

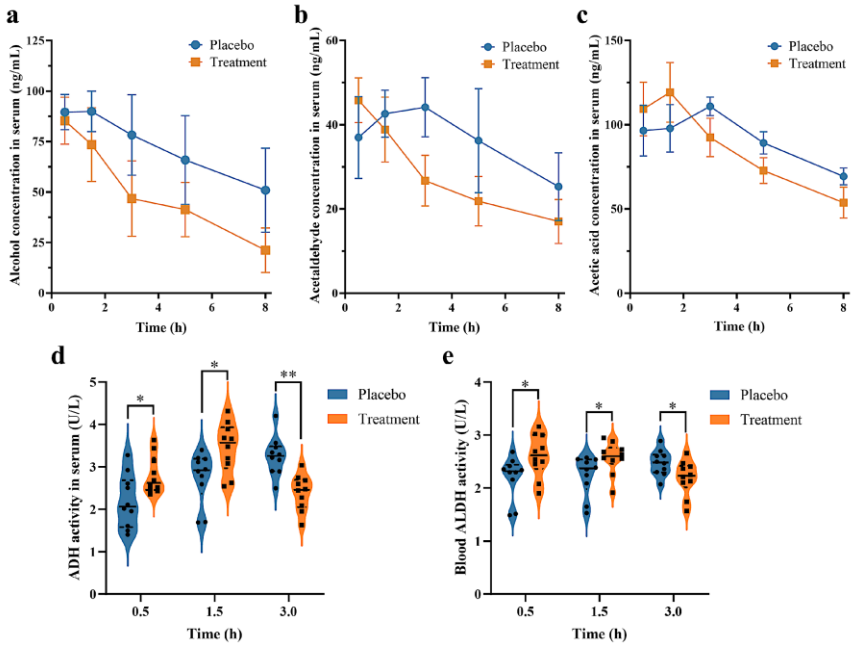


Figure 4. The regulatory effect on ethanol metabolism of HBF. (a) Serum alcohol concentration; (b) Serum acetaldehyde concentration; (c) Serum acetic acid concentration. (d) The change of blood ADH activity; (e) The change of blood ALDH activity. *, $p < 0.05$; **, $p < 0.01$.

Hepatotoxicity Assessment of HBF: The activity of ALT and AST were determined as shown in Figure 5. The consistently lower ALT levels in the treatment group suggested that HBF may attenuate ethanol-induced hepatocellular injury. At 3 h, the treatment group had significantly ($p < 0.01$) lower ALT activity (36.71 U/L) than the placebo group (43.66 U/L). Treatment group consistently had lower AST activity at all time points compared to the placebo group. However, there was no statistically significant difference between these two groups.

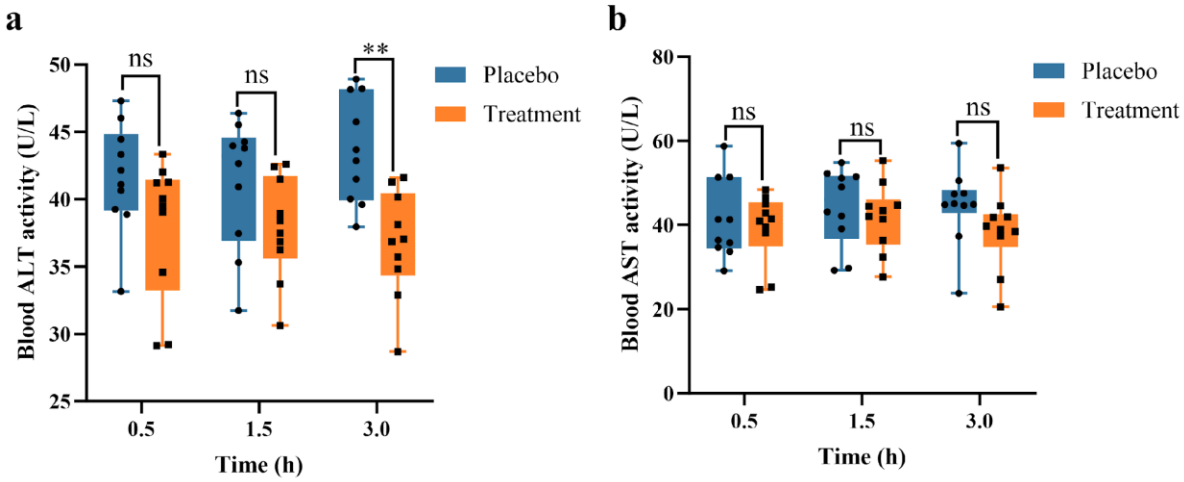


Figure 5. The hepatoprotective effects of HBF. (a) The effects of HBF on ALT activity of blood. (b) The effects of HBF on AST activity of blood. **, $p < 0.01$; ns, no significance.

Antioxidant and Anti-inflammatory Effects of HBF: Figure 6 a–c illustrate the change of oxidative stress in blood at three different time points (0.5h, 1.5h, and 3h) after drinking. The MDA content in treatment group (3.28–3.43 nmol/mL) was reduced compared to placebo group (3.47–3.61 nmol/mL) between 0.5 h and 3 h. The SOD activity for the treatment group at 0.5h (239.64 U/L) and 1.5h (210.23 U/L) was higher than the placebo group, which had values of 205.19 U/L and 178.97 U/L, respectively. At 3h, the treatment group showed a slight reduction in SOD activity (193.23 U/L) compared to the placebo group (212.85 U/L). The GPx activity ranged from 120.58 U/L to 136.25 U/L in placebo group. Similarly, the GPx activity in blood was 125.05–131.39 U/L after HBF treatment.

Moreover, we measured the levels of inflammatory factors in the volunteer blood (Figure d, e). At 0.5, 1.5, 3h, the levels of TNF-α in blood of participants in the placebo group were 1.88 μg/L, 2.20 μg/L, and 2.39 μg/L, respectively. The levels of TNF-α in blood of participants in the treatment group were 1.34 μg/L, 1.43 μg/L, and 1.51 μg/L, respectively. In general, administration of HBF reduced the levels of TNF-α and showed a very significant difference ($p < 0.01$). At 0.5h and 3h, the treatment group showed significantly ($p < 0.05$) lower IL-1β levels compared to the control group. Although the IL-1β concentration of the treatment group (23.13 μg/L) in the blood was slightly higher than that in the placebo group (22.40 μg/L) at 1 h, no statistically significant difference was observed between them.

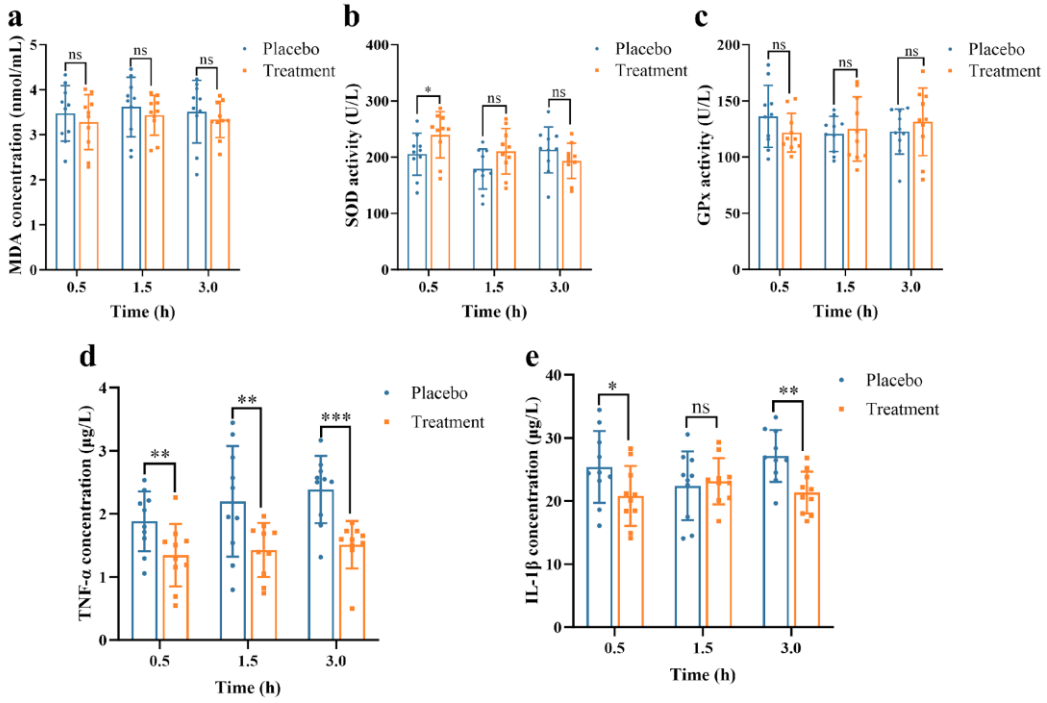


Figure 6. Effects of HBF on oxidative stress and inflammatory cytokine levels in blood. (a) MDA concentration; (b) SOD activity; (c) GPx activity. (d) TNF-α concentration; (e) IL-1β concentration. Oxidative stress markers: (a), (b) and (c); Inflammatory cytokines: (d) and (e). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, no significance.

DISCUSSION

Complex dietary interventions are becoming a novel paradigm for employing functional foods in disease prevention and treatment [22, 23]. Alcohol metabolism disorder is considered a key factor contributing to alcohol-induced hangovers [24]. It has been reported that rapid alcohol clearance is associated with lower hangover severity and incidence [25]. Currently, anti-hangover products on the market mainly consist of plant extracts and other active substances, with some products relying on probiotics. However, their effectiveness and safety still lack specific scientific evidence. This study innovatively develops HBF, an anti-hangover formula, combining plant extracts and probiotics, and investigates its effectiveness and safety in healthy males. The selected participants were divided into two groups: a placebo group and a treatment group, with 10 volunteers in each group who complete the experiment. Additionally, there are no significant differences between the two groups in terms of physical characteristics and lifestyle habits, which ensures the reliability of the final experimental results.

Pueraria lobata is rich in isoflavones, including daidzein, genistein, and puerarin. These active substances can regulate the activity of ADH and ALDH, thereby aiding in ethanol metabolism [26]. *Hovenia dulcis* has been proven to help liver detoxify by enhancing the activity of ALDH, reducing inflammation and oxidative stress [27]. *Hovenia dulcis* water extracts, containing 15% flavonoid, have been demonstrated to improve acute alcoholism and liver injury [28]. The key active substance involved anti-hangover is dihydroquercetin [29]. Probiotic-based alcohol detoxification is gradually emerging as a novel approach to relieving alcohol intoxication. *Lactobacillus rhamnosus* GG exerts hepatoprotective effects and helps prevent acute alcohol-induced hepatic steatosis and liver injury [30]. Similarly, *Lactobacillus plantarum* CMU995 can inhibit the translocation of alcohol-derived endotoxins to the liver, further alleviating alcohol-induced liver injury in

mice [31]. The volunteers received the treatment of 5g HBF in treatment group. 5g HBF contained 624.3 mg of polyphenols, 450.6 mg of flavonoids, which were sourced from *Pueraria lobata* and *Hovenia dulcis* water extracts. In addition, it also contained a certain amount of *Lactobacillus rhamnosus* Alcoker0067 and *Lactobacillus plantarum* ZQ15 domesticated by our laboratory.

Alcohol and its intermediate metabolite, acetaldehyde, are considered harmful to the human body. Acetaldehyde has a more direct impact on hangover among them. Acetaldehyde is a highly reactive aldehyde compound that readily forms covalent bonds with intracellular macromolecules such as proteins and DNA, leading to functional disturbances [32]. Acetaldehyde is capable of crossing the blood–brain barrier, where it exerts neurotoxic effects on the central nervous system, contributing to the exacerbation of hangover-related symptoms [33]. In this study, we assessed serum alcohol, acetaldehyde and acetic acid concentrations to evaluate the potential hangover-relieving effect of HBF. The alcohol levels in serum showed a decrease in the HBF group compared to the placebo group at all time points, indicating HBF can accelerate alcohol metabolism and eliminate acetaldehyde. This was further supported by the observation that serum concentrations of acetaldehyde and acetate in the treatment group continuously decreased from 0.5 h to 8 h and remained at lower levels compared to the placebo group. Moreover, the serum activities of ADH and ALDH at 0.5 h and 1.5 h were significantly ($p < 0.05$) higher in the treatment group compared to the placebo group. These findings indicate that HBF can enhance the enzymatic activities of ADH and ALDH.

Excessive alcohol consumption usually leads to the elevated levels of AST and ALT, indicating potential liver injury [34]. HBF treatment can decrease the activities of AST and ALT in serum. This suggests a potential hepatoprotective effect in preventing alcohol-induced liver injury. Excessive alcohol intake can induce oxidative stress by increasing the levels of potent oxidants such as reactive oxygen species (ROS), which damage cellular macromolecules and lead to hepatocellular injury [35]. Antioxidant enzymes such as CAT, SOD, and GPx serve as key indicators for assessing oxidative stress levels. While MDA, as a byproduct of lipid peroxidation, also can be used as a reliable biomarker of oxidative stress. The levels of MDA in treatment group were lower than that in placebo group. And HBF improved SOD activity in the blood of the treatment group, with a particularly notable difference ($p < 0.05$) observed at 0.5 h. However, no significant difference in GPx activity was observed between the two groups. Alcohol can lead to the accumulation of inflammatory factors, which causes contributing to the development of alcoholic liver disease [36]. And the inflammatory response plays a critical role in the pathophysiology of alcohol-induced hangovers [37]. The levels of pro-inflammatory cytokine TNF-α in the placebo group was significantly ($p < 0.01$) higher than that in the treatment group. Although the IL-1β levels in the blood of the treatment group were slightly higher than those of the placebo group at 1.5 h, no significant difference was observed between the two groups. However, at 0.5 and 3 h, the IL-1β levels in the treatment group were significantly ($p < 0.05$) lower than those in the placebo group. Therefore, HBF markedly alleviates alcohol-induced oxidative stress and mitigates the associated inflammatory response. Effective antioxidant activity is regarded as a fundamental attribute required for functional foods [38].

CONCLUSIONS

This randomized controlled trial demonstrated that the novel herbal–probiotic formula (HBF) significantly enhances alcohol metabolism by promoting ADH and ALDH activities, accelerating the clearance of ethanol and acetaldehyde, and increasing acetate production. In addition, HBF exhibits hepatoprotective and antioxidant

properties, as evidenced by reduced ALT and MDA levels and increased SOD activity. The HBF also alleviates alcohol-induced inflammation, as reflected by lower levels of TNF-α and IL-1β. These findings suggest that HBF is a promising for mitigating hangover symptoms and protecting against alcohol-related liver damage. Furthermore, when viewed through the FFC model, the case of berberine illustrates how a bioactive compound can progress through stages of identification, mechanistic validation, and preliminary clinical evidence, while still requiring large-scale clinical trials and regulatory approval. Similarly, although our results provide encouraging evidence in anti-hangover. Additional experiments, including expanded clinical validation and long-term safety assessments, are necessary before HBF can be fully established as a functional food.

Abbreviations: ADH: Alcohol dehydrogenase, ALDH: Aldehyde dehydrogenase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, FFC: Functional Food Center, GPX: Glutathione peroxidase, HBF: Herbal-probiotic formula, IL-1β: Interleukin-1 beta, MDA: Malondialdehyde, SOD: Superoxide dismutase, TNF-α: Tumor necrosis factor-alpha.

Competing Interests: The authors declare that they have no known competing financial interests with any individual or organization.

Author Contributions: Writing—original draft preparation, C. S.; writing—review and editing, F. L.; visualization, H. Z.; supervision, P. Z.; methodology, Y. Z.; data curation, L. J.; investigation, T. Z.; formal analysis, L. C.; funding acquisition and project administration, Q. L.; conceptualization, D. L. All authors have read and agreed to the published version of the manuscript.

Acknowledgments and funding: This work was supported by Key Research and Development Program of Henan Province (241112110900) and Key Research and Development Program of Henan Province (241112110700).

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