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Baijiu hangover: Correlation analysis between neurobiochemical and behavioral parameters in a mouse model and clinical symptoms

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ABSTRACT

Baijiu, a distilled Chinese spirit, is one of the most popular alcoholic beverages. However, excessive drinking of Baijiu may cause severe hangover symptoms, including headache, reduced appetite, and dizziness. Herein, we aimed to characterize various clinical hangover symptoms induced by binge consumption of Baijiu samples with different post-drinking qualities in a BALB/c mouse model. Baijiu sample with higher concentrations of alcohols and aldehydes resulted in more severe hangovers in population. Mice administered with the low post-drinking quality Baijiu exhibited altered neurobiochemical parameters and impaired motor performance 10 h after gavage dose of 4.3 g ethanol/kg body weight, including decreased brain 5-hydroxytryptamine, sucrose preference, increased serum endothelin and gait ataxia. Correlation analysis further revealed that these mouse indices were strongly correlated with hangover symptoms in population, and profound correlations were observed between mice indicators and Baijiu congeners. These results indicated that assessing these mouse indices at 10 h after administration can effectively discriminate Baijiu with different hangover effects, and high concentrations of congeners, such as alcohols and aldehydes, may be responsible for the varying post-drinking qualities of Baijiu.

1. Introduction

Alcohol hangover is characterized by a combination of various unpleasant physiological and psychological experiences that occur after consuming a large amount of alcohol. These symptoms typically arise several hours after drinking, particularly when the blood alcohol concentration (BAC) approaches zero (Verster et al., 2010, 2020). Alcohol hangovers can last for several hours or even more than 24 h, and research has identified 47 symptoms associated with this condition (Penning et al., 2012), with the most common being headache, dizziness, fatigue, nausea, loss of appetite, and impaired attention (Van Schrojenstein Lantman et al., 2010). Moreover, the pain-related effects of ethanol, such as headache and hyperalgesia, are evident, particularly during the onset of hangovers (Jochum et al., 2010). Owing to neurocognitive impairments related to executive function, such as impaired attention, memory and psychomotor skills, hangover is associated with a range of medical and socioeconomic problems (Gunn et al., 2018). Hangover can pose a threat to health, as it increases blood pressure and heart rhythm disturbances, which can cause sudden death (Razvodovsky, 2021), and the annual economic losses due to decreased productivity associated with hangover syndrome are about $179 million in the United States (Stephens et al., 2010).

Baijiu, an ancient Chinese distilled spirit, is a clear and transparent fermented alcoholic beverage with a high ethanol content, ranging from 38% to 65% by volume. Baijiu is the national liquor of China and has a unique position in traditional Chinese culture (Liu & Sun, 2018). Additionally, Baijiu is a major component of the Chinese food and beverage industry, with global consumption exceeding 13 billion liters,

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representing a market value of approximately $97.4 billion (Hong et al., 2023). However, binge consumption of Baijiu and other alcoholic beverages may cause severe hangover symptoms such as headache, nausea, and dizziness (Wang et al., 2016), leading to concerns about Baijiu consumption. Besides the toxic effects of ethanol, congeners such as higher alcohols, biogenic amines and aldehydes have also been linked to intoxication and hangovers, because they may intensify the toxic effects of ethanol in alcoholic beverages (Krymchantowski & da Cunha Jevoux, 2014).

Existing evaluations of hangover severity are primarily based on the descriptions of the population. Rohsenow et al. combined the symptoms mentioned in previous studies to compose a new list of symptoms, the Acute Hangover Scale (Rohsenow et al., 2014). Other researchers also raised different assessment indicators of alcohol hangover (Penning et al., 2013; Robertson et al., 2011). After comparison of these indicators, Hogewoning et al. proposed a hangover severity evaluation model consisting of 23 individual symptoms, such as headache, dizziness and reduced appetite, providing a comprehensive overview of hangover status (Hogewoning et al., 2016). However, the results of these surveys are subjective and might be affected by individual differences, and overconsumption of alcoholic beverages could also cause health and ethical problems that impede population experiments. Therefore, an evaluation method should be developed based on animal experiments. However, evaluating these symptoms in experimental animals proved challenging, most studies focusing on the effects of pure ethanol rather than alcoholic beverages. Karadayian et al. reported the development of an intraperitoneal injection mouse hangover model, which utilized BAC to determine the onset of alcohol-induced hangover (less than or equal to 10% of the maximum BAC, Karadayian et al., 2012). Based on this model, impairments in motor function and affective behavior were investigated in hangover mice after 6 h of ethanol administration (Karadayian & Cutrera, 2013; Karadayian et al., 2013). A range of neurobiochemical parameters are altered during hangover stage, and the intensity of these alterations could reflect the severity of hangovers. Acute ethanol consumption affects various neurotransmitter systems, including dopamine, serotonin (5-HT), gamma-aminobutyric acid, and glutamate (Palmer et al., 2019). The stimulating effects observed during initial alcohol intoxication are associated with alterations in dopamine levels and brain-derived neurotrophic factors (Bosse et al., 2012). Brain-derived neurotrophic factors promote the activation of the tropomyosin receptor kinase B receptors and downstream signaling pathways (Tanaka et al., 2014). In experimental animals, hypoactivity, anxiety-like behavior, and reduced wheel-running activity are observed during hangovers (Bustamante et al., 2012; Santos et al., 2020). Additionally, previous studies have demonstrated that mice experience a decline in motor performance during the initial stages of alcohol hangover. This reduction in motor function has been linked to alterations in brain cortex energetic metabolism (Karadayian et al., 2012, 2013).

In this study, a mouse model was established to evaluate the hangover effects after heavy Baijiu consumption. Mice neurobiochemical parameters, motor performance, and other indicators were assessed to evaluate hangover effects of Baijiu samples. Correlation analyses were performed to investigate their correlations with hangover symptoms of Baijiu in population, and the relations between key indices and main component of Baijiu. This study provides a method for evaluating the hangover effects of Baijiu and may help in the identification of compounds responsible for hangovers in alcoholic beverages.

2. Materials and methods

2.1. Reagents and samples

Standards of main volatile compounds were obtained from Sigma Aldrich (Shanghai, China), including ethanol, n-propanol, isobutanol, butanol, isoamyl alcohol, 2-phenylethanol, ethyl acetate, ethyl hexanoate, ethyl dodecanoate, isoamyl n-butyrate, and isoamyl caproate. Lactic acid and acetic acid were purchased from Shanghai Aladdin Bio-Chem Technology Co., Ltd (Shanghai, China). Ultrapure water was generated with a Milli-Q system (Millipore, Bedford, MA, USA).

Edible ethanol (98% by volume) was obtained from Azelis Co., Ltd (Shanghai, China), and it was diluted to 45% with ultrapure water for both population and animal experiment. Two luzhou-flavor Baijiu samples with same alcohol content (45% by volume) were purchased from local market, including Baijiu A and Baijiu B. The flavor compounds and organic acids in Baijiu samples were analyzed by headspace solid phase microextraction (HS-SPME) combined with GC-MS (Thermo Fisher Scientific, Massachusetts, USA) and HPLC (Waters Co., Milford, USA), according to previous reports (Wu et al., 2017; Zhang et al., 2022), and the results were shown in Table S1.

2.2. Evaluation of hangover effect of Baijiu samples

A clinical observation test was conducted on male participants to evaluate the hangover effect of Baijiu samples by describing the hangover symptoms in the morning following beverage administration. A naturalistic study approach was adopted to ensure mimicking of real-life drinking. The study protocol was approved by the Psychology Ethics Committee of Jiangnan University (reference number: JNU20230301IRB04).

2.2.1. Participants

All participants must be at least 21 years of age and meet the following criteria:

(1) No serious drinking problems (scored <5 on the Short Michigan Alcohol Screening Test) and no history of treatment or counseling for alcohol problems (Devenney et al., 2019).
(2) Five or more drinks on a single occasion at least once in the 30 days prior to screening.
(3) No health problems or current medication use contraindication for alcohol.
(4) Fluent expression.
(5) Prior to beverage administration, participants should not consume alcohol, caffeine, prescription or over-the-counter drugs within the prior 24 h, or food or beverage within the prior 3 h.

2.2.2. Procedure

Alcoholic beverages were administered to yield a hangover state in participants (1.1 g ethanol/kg body weight (bw) for men, Howland et al., 2010). Beverages were served in small groups of 5 participants between 8:30 p.m. and 10:00 p.m. After drinking and a 30-min absorption period, participants were escorted to rooms where they had an 8-h bed rest and observed by nursing students. In the morning, participants were awakened, ate breakfast, and then completed the hangover ratings and other questionnaires. Research staff were responsible for observing the participants in case of emergency and providing necessary instruction.

2.2.3. Evaluation of hangover symptoms

A questionnaire consisting of 24 individual hangover symptoms was completed by the participants to evaluate the hangover symptoms (Hogewoning et al., 2016; Penning et al., 2013). The symptoms were rated on an 11-point scale ranging from 0 (absent) to 10 (extreme).

2.3. Animal experiments

Specific pathogen free (SPF) wild-type BALB/c mice (male, aged 7−8 week and weighed 20−24 g) were obtained from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The mice (n = 150) were housed in a controlled environment at 22 ± 2 °C, 40%–60% humidity, and a 12-h light/dark cycle (SPF, SYXK 2021-0056). Mice had access to

water and fodder ad libitum, and their body weight and general health were closely monitored. The experimental protocol complied with the license of Animal Management and Use Committee of Jiangnan University (reference number: JN. No 20230615b1000709).

The procedure of animal experiments was visualized in Fig. 1a. After a one-week acclimatization period, mice were distributed to two experiments including the gavage dose optimization and the hangover indices study. All the mice went through a fasting period of 6 h before experiments. In the gavage dose optimization experiment, 45 mice were randomly divided into 9 treatment groups (n = 5). Three samples were included (edible alcohol and two Baijiu samples) and each sample was administered at three gavage doses. The gavage doses of 8, 12 and 16 mL/kg bw (namely 2.9, 4.3 and 5.8 g ethanol/kg bw) were selected according to previous reports on alcohol hangover of mice, which ranged from 2 g ethanol/kg bw to 8 g ethanol/kg bw (Chen et al., 2014; Hori et al., 2003; Karadayian et al., 2013). After gavage, the intoxication time and sober time of mice were accessed by the appearance and recovery of loss of righting reflex (LORR, Gao & Calderon, 2020), and the intoxication rate along with mortality of mice were recorded. Additionally, small volume of blood sample was collected from mice by tail cut at 2, 4 and 6 h. Then the mice were given fodder and water to rehabilitate for 48 h before receiving sucrose preference test (SPT) with extra 5 mice as blank control. At the end of the experiment, mice were sacrificed after isoflurane anesthesia (2% by volume) in an inhalation anesthetic apparatus. In the hangover indices study, 100 mice were randomly divided into 20 treatment groups (n = 5). Mice were administered with four samples including 0.9% saline, alcohol, HB and LB at the gavage dose of 12 mL/kg bw. After gavage, five mice in each group were sacrificed at corresponding time point (6, 8, 10, 12 and 14 h) and blood was drawn from the orbit after isoflurane anesthesia (2% by volume). Blood samples were allowed to clot for 2 h at room temperature before centrifugation for 15 min at 1000×*g*. Supernatants were then collected to carry out the serum related indicators. Brain tissue of mice was collected then frozen in liquid nitrogen, and restored in −80 °C.

2.4. Measurements of neurobiochemical parameters

2.4.1. Serum

Serum ethanol was measured using 20 μL of serum sample by an ethanol detection kit (Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's protocol. Serum prostaglandin E2 (PGE2), histamine (His), 5-hydroxytryptamine (5-HT), endothelin (ET), antidiuretic hormone (ADH) and calcitonin gene related peptide (CGRP) levels were quantified using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's protocol (Meibiao Biotechnology, Jiangsu, China), small aliquot of 10 μL of serum sample was used for each index.

2.4.2. Brain tissue

Brain tissue samples were thawed and homogenized in 5% (v/v) perchloric acid solution (w:v = 1:9), after centrifugation at 12000×*g* for 10 min, and the supernatant was filtered through a 0.22 μm glass filter and stored at 4 °C for measurement (Tian, Wang, et al., 2019). Brain histamine, 5-HT, and dopamine (DA) levels were determined in aliquot

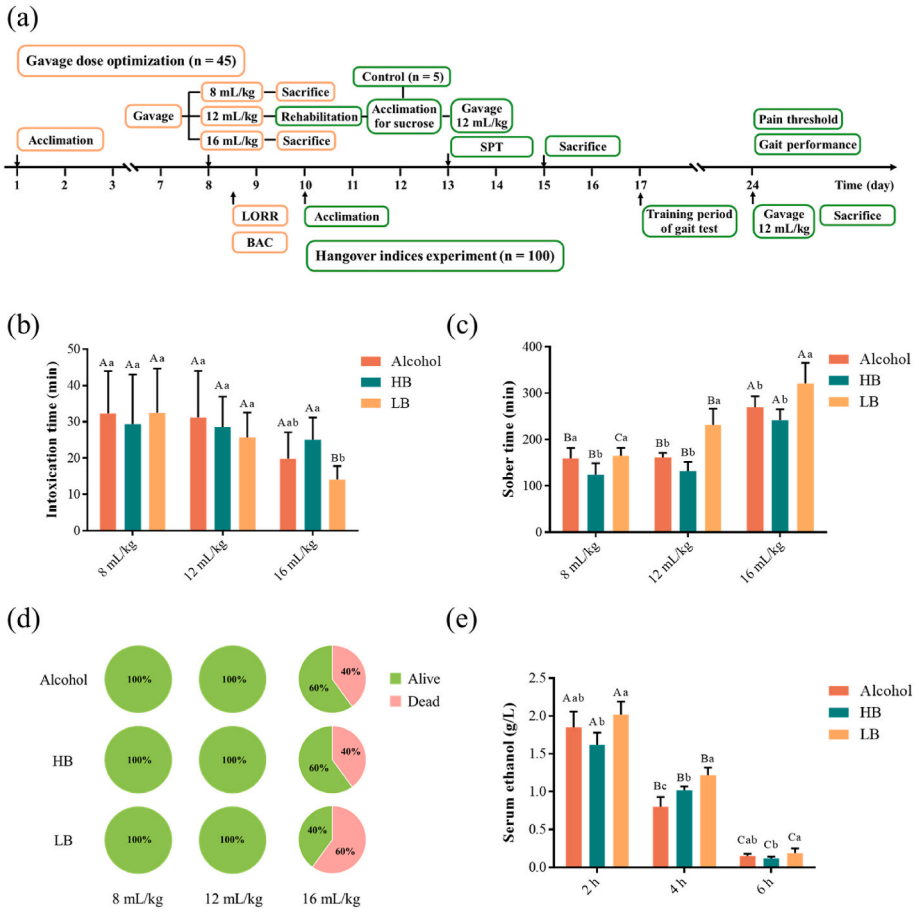

Fig. 1. Effects of different gavage doses on mice. (a) Timeline of animal experiments, (b) intoxication time, (c) sober time, (d) livability and (e) serum ethanol concentration under gavage dose of 12 mL/kg bw. Different uppercase letters (A, B, C) represent significant ($p < 0.05$) differences between different gavage doses or time after gavage (for the same alcoholic beverage sample), and different lowercase letters (a, b, c) represent significant ($p < 0.05$) differences between different alcoholic beverage samples (at the same gavage dose or time after gavage).

of 10 μL of brain homogenate by ELISA kits according to the manufacturer's protocol (Meibiao Biotechnology, Jiangsu, China).

2.5. Pain threshold detection

The hyperalgesia of mice during hangover state was evaluated by pain threshold examination (Nan et al., 2022; Zhang et al., 2017). A von Frey pain threshold detector (Woodland Hills, CA, USA) was applied perpendicularly to the periorbital and pelma regions of the mice to obtain pain thresholds. A positive response on the von Frey test was documented when paw retraction occurs or the mice stroked its face with the ipsilateral forepaw, and the head recoiled quickly toward the side away from the stimulus or vocalization.

2.6. SPT

The SPT was performed in mice after Baijiu administration (Zhang et al., 2017). At the start of the experiment, the animals were trained to adapt a 2% sucrose solution by exposing them to sucrose in two bottles for 24 h. Then one of the bottles was replaced with water and the mice were trained for another 24 h. After the acclimation period, the mice went through a 24-h food and water deprivation, then each animal was presented simultaneously with two weighed bottles, one contained 2% sucrose solution and the other contained water. The two bottles were reweighed 24 h later, and the percent preference for sucrose consumption was calculated. Sucrose preference (%) = sucrose solution consumption/(sucrose solution consumption + water consumption) × 100.

2.7. Evaluation of mice gait

Catwalk XT gait analysis system (Noldus, Wageningen, The Netherlands) was used to monitor the gait of mice to assess motor performance of hangover mice (Pitzer et al., 2021; Tatenhorst et al., 2016). During the training stage, mice were habituated to the CatWalk XT system and trained to cross the corridor voluntarily for three accomplished runs without stopping, turning around, or changing direction. In the test stage, different groups of mice were intragastric administration with corresponding samples, then placed in the walkway, and videotaped from below. Footprints were automatically recorded by the Catwalk XT 10.0 software, and detection settings were as follows: Camera gain 20; intensity threshold 0.10; maximum allowable speed variation 60%. For the analysis, gait parameters were automatically generated after each footprint being manually checked and respectively labeled LF (left front), LH (left hind), RF (right front), and RH (right hind) paws. These parameters include body and swing speeds, stand and swing times, print area, stance, propelling, stride length, stride frequency, paw angle, gait symmetry, ataxia coefficient, and overlap distance.

2.8. Statistical analysis

The results were reported as mean ± standard deviation (SD). An analysis of variance (ANOVA) accompanied by post-hoc multiple comparisons (Tukey's test) was performed to determine the significance of differences using IBM SPSS Statistics Version 19.0 (IBM Corp., Armonk, NY) with a significance level of 0.05. Differences between composition of Baijiu samples were analyzed using orthogonal partial least squares discriminant analysis (OPLS-DA) model, and Spearman correlation analysis was performed by R (version 4.1.0) packages.

3. Results

3.1. Clinical hangover symptom evaluation of Baijiu samples

The evaluation of Baijiu samples involved a total of 15 participants. The demographic information of the participants, including age, height, and weight, is presented in Table S2. No obvious difference was observed among the alcohol, Baijiu A, and Baijiu B groups. The overall hangover severity of edible alcohol, Baijiu A, and Baijiu B, which was evaluated using the one-item score, was shown in Table 1. After binge consumption, the one-item hangover severity of Baijiu B was significantly higher than that of the edible alcohol and Baijiu A ($p < 0.05$). Additionally, the scores of 24 hangover symptoms were recorded by the participants on the second morning (Table 1). Hangover scores for symptoms related to thirst and drowsiness, such as sleepiness and tiredness, were highest among all the hangover symptoms. However, no remarkable differences were observed among the three groups in terms of these three items. Symptom severity scores for most symptoms (15 out of 24) reported by the Baijiu A group were significantly lower ($p < 0.05$) than the those of the Baijiu B group, including headache, dizziness, reduced appetite, and reduced alertness.

Participants in Baijiu A group also exhibited a significantly ($p < 0.05$) lower hangover severity than the alcohol group in terms of motor performance, mood-related symptoms, and other daily activities, such as dizziness, clumsiness, nausea, reduced appetite, and regret. Additionally, the Baijiu A group also showed mild symptoms, such as apathy, increased heartbeat, and sensitivity to light. Generally, the Baijiu A group displayed the lowest hangover severity, followed by the alcohol and Baijiu B groups. According to the population evaluation results, the Baijiu A and Baijiu B were determined to be high post-drinking quality Baijiu (HB) and low post-drinking Baijiu (LB), respectively.

3.2. Optimal gavage dose of Baijiu samples

The intoxication time, sober time, and livability of the mice at different gavage doses were measured to determine the optimal dose. Reduced intoxication time of alcohol, HB, and LB groups was observed with the increase in gavage dose, and no obvious differences were observed among the three groups at 8 mL/kg bw and 12 mL/kg bw. The shortest intoxication time was observed in the LB group at 16 mL/kg bw, which was significantly ($p < 0.05$) shorter than that in the HB group (14.13 *vs.* 25.06 min, Fig. 1b). Sober time increased with an increase in the gavage dose, and a significantly longer sober time was observed in

Table 1
Hangover symptom severity of different baijiu samples. Different lowercase letters (a, b, c) represent significant ($p < 0.05$) differences among different groups of participants in each row.

Hangover items	Mean Score ± SD		
	Alcohol group	Baijiu A group	Baijiu B group
One-item hangover score	4.0 ± 0.9b	3.0 ± 1.3b	5.8 ± 1.0a
Sleepiness	4.8 ± 1.7a	4.8 ± 1.6a	5.6 ± 1.4a
Tiredness	4.2 ± 0.7a	4.2 ± 1.1a	5.0 ± 0.9a
Thirst	7.0 ± 1.7a	4.2 ± 3.0a	6.8 ± 1.5a
Headache	3.4 ± 0.8b	1.3 ± 1.1b	3.8 ± 0.7a
Concentration problems	3.2 ± 1.2 ab	1.7 ± 1.7b	4.0 ± 1.8a
Reduced alertness	3.0 ± 1.2 ab	1.6 ± 1.7b	3.7 ± 1.8a
Nausea	4.2 ± 1.9a	1.3 ± 1.2b	3.2 ± 1.0 ab
Weakness	4.0 ± 1.7a	2.0 ± 1.2b	3.8 ± 1.2 ab
Dizziness	5.2 ± 1.2a	1.5 ± 1.0b	4.4 ± 1.9a
Clumsiness	3.8 ± 1.2a	1.3 ± 0.7b	3.8 ± 1.0a
Stomach pain	0.0 ± 0.0b	0.0 ± 0.0b	3.6 ± 1.6a
Apathy	1.6 ± 0.8b	0.0 ± 0.0c	3.6 ± 1.7a
Shaking, shivering	0.2 ± 0.4b	0.0 ± 0.0b	2.0 ± 0.9a
Regret	2.2 ± 0.7b	0.8 ± 0.7c	3.8 ± 1.2a
Reduced appetite	3.2 ± 0.7a	1.2 ± 0.9b	4.4 ± 1.5a
Heart beating	5.4 ± 1.6a	2.0 ± 1.2b	4.6 ± 1.9a
Vomiting	1.0 ± 0.6b	1.2 ± 0.9b	5.2 ± 1.0a
Confusion	2.8 ± 2.3 ab	0.7 ± 1.1b	3.4 ± 2.0a
Sensitivity to light	2.2 ± 1.2a	0.0 ± 0.0b	2.6 ± 1.9a
Sleep problems	4.2 ± 1.8a	1.5 ± 1.4b	2.8 ± 1.8 ab
Heart racing	4.2 ± 1.0a	2.8 ± 1.2a	3.8 ± 1.2a
Sweating	4.6 ± 1.9a	2.3 ± 1.9a	3.6 ± 1.9a
Anxiety	1.6 ± 1.4a	1.0 ± 0.8a	2.4 ± 1.0a
Depression	0.6 ± 0.8b	0.2 ± 0.4b	1.6 ± 0.5a

all three groups at 16 mL/kg bw than that at 8 and 12 mL/kg bw ($p < 0.05$). The sober time of HB group ranged from 124.50 to 241.49 min, which was significantly ($p < 0.05$) shorter than that of the LB group (164.84–321.15 min) and alcohol group (159.32–269.70 min) at all the three same gavage doses (Fig. 1c).

At a dose of 16 mL/kg bw, the death rate was 40% for mice in the alcohol and HB groups and 60% for mice in the LB group. All mice in the three groups survived at doses of 8 and 12 mL/kg bw, which were considered non-lethal gavage doses (Fig. 1d). Regarding the intoxication rate, doses of 12 and 16 mL/kg caused complete intoxication in all three groups; however, only 60% of mice in the alcohol and HB groups and 80% in the LB group showed signs of intoxication at dose of 8 mL/kg bw (Fig. S1). Additionally, the serum ethanol levels of mice were measured at 2, 4, and 6 h after gavage under the dose of 12 mL/kg bw. The values for all three groups at 2 h were approximately 2 g/L and decreased to less than 0.2 g/L at 6 h (Fig. 1e), consistent with the criteria for determination of mouse hangover onset. Overall, based on the moderate intoxication and sober time and fine livability observed in mice, the optimal gavage dose for Baijiu hangover investigation was determined to be 12 mL/kg bw, equivalent to 4.3 g ethanol/kg bw.

3.3. Neurobiochemical parameters of hangover mice

Neurobiochemical parameters, including histamine, endothelin, dopamine, and 5-HT levels, were measured in the serum and brain tissue of mice following the administration of different Baijiu samples. The results were presented in Fig. 2.

3.3.1. Serum neurobiochemical parameters

Compared with the control group (16.70 ng/L), serum histamine levels were observed to be significantly ($p < 0.05$) lower at 8 h after gavage with alcohol, HB, and LB, all measured below 14.46 ng/L. Additionally, mice in the LB group showed significantly ($p < 0.05$) lower serum histamine levels than those in the other three groups at 10 h after administration and returned to control level at 12 h. No obvious differences in serum histamine levels were found among the four groups at 6, 12, and 14 h after gavage.

The administration of alcoholic samples remarkably increased the serum endothelin concentration in mice. LB group exhibited significantly ($p < 0.05$) higher serum endothelin levels (44.83 ng/L) than the control and alcohol groups (40.65 and 40.20 ng/L, respectively) 6 h after gavage. A similar trend was observed at 8 h after administration. The serum endothelin concentrations in mice after gavage for 10 h were in the following order: Control < HB < alcohol < LB, and the differences between each two groups were significant ($p < 0.05$). Serum endothelin in the alcohol and HB groups returned to normal levels after 12 h of administration, while the endothelin level in the LB group remained higher than that in the control group.

3.3.2. Brain neurobiochemical parameters

The brain 5-HT concentration in mice in the alcoholic group was lower than that in the normal control group during hangovers. At 6 h, the LB group already displayed significantly ($p < 0.05$) lower 5-HT than the control group (183.85 *vs.* 243.89 ng/L). When the time was extended to 8 and 10 h, all three alcoholic groups showed significantly ($p < 0.05$)

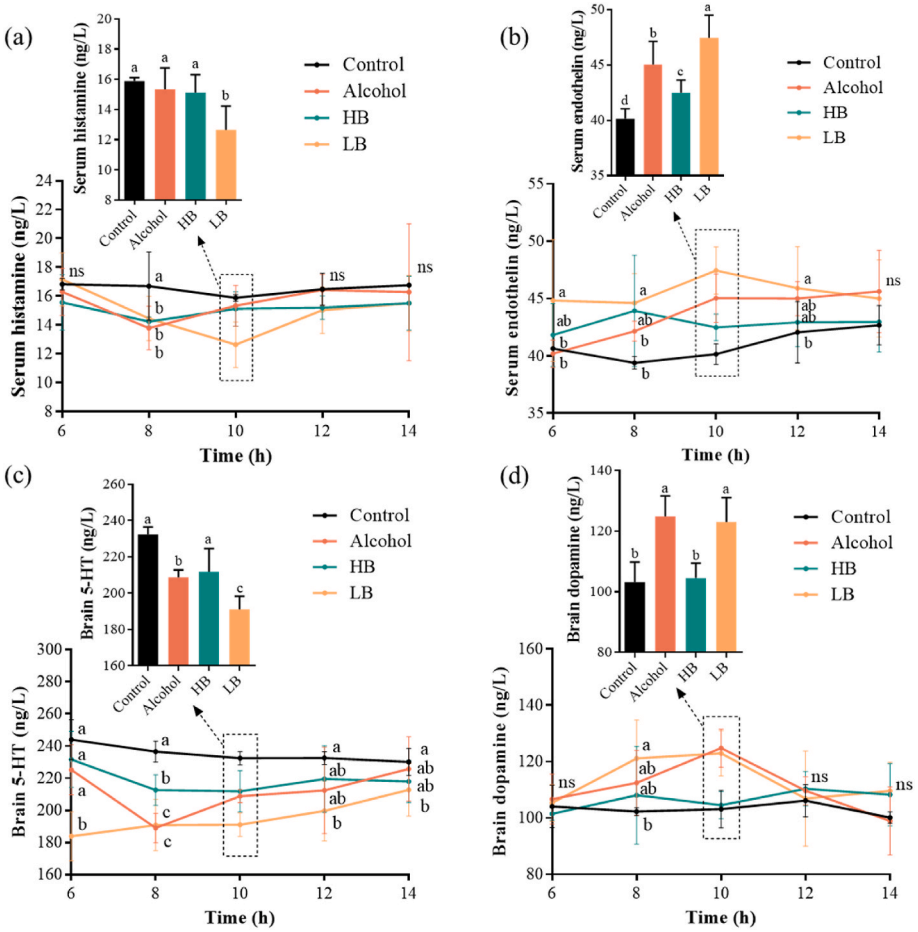

Fig. 2. Neurobiochemical parameters of mice after gavage. (a) Serum histamine, (b) serum endothelin, (c) brain 5-HT and (d) brain dopamine. Different lowercase letters (a, b, c) represent significant ($p < 0.05$) differences between different groups of mice at the same time, and ns represent no significant differences between all the four groups.

lower 5-HT levels than the control group. Nevertheless, obviously higher 5-HT were observed in the HB group than that in the LB and alcohol groups at these two time points. At 12 h, the brain 5-HT concentration of mice in the alcohol group began to return to control level, and no significant differences were found among the control, alcohol, and HB groups ($p > 0.05$).

Administration of alcohol and LB resulted in an increase in brain dopamine concentration at 8 and 10 h after gavage. A significantly ($p < 0.05$) higher brain dopamine concentration was found in the LB group than that of the control group (121.15 *vs.* 102.29 ng/L). At 10 h after administration, the dopamine levels exhibited significantly ($p < 0.05$) higher in both alcohol (124.80 ng/L) and LB (123.00 ng/L) groups compared with that of the control (103.15 ng/L). Notably, no significant differences were observed between the control and HB groups during the

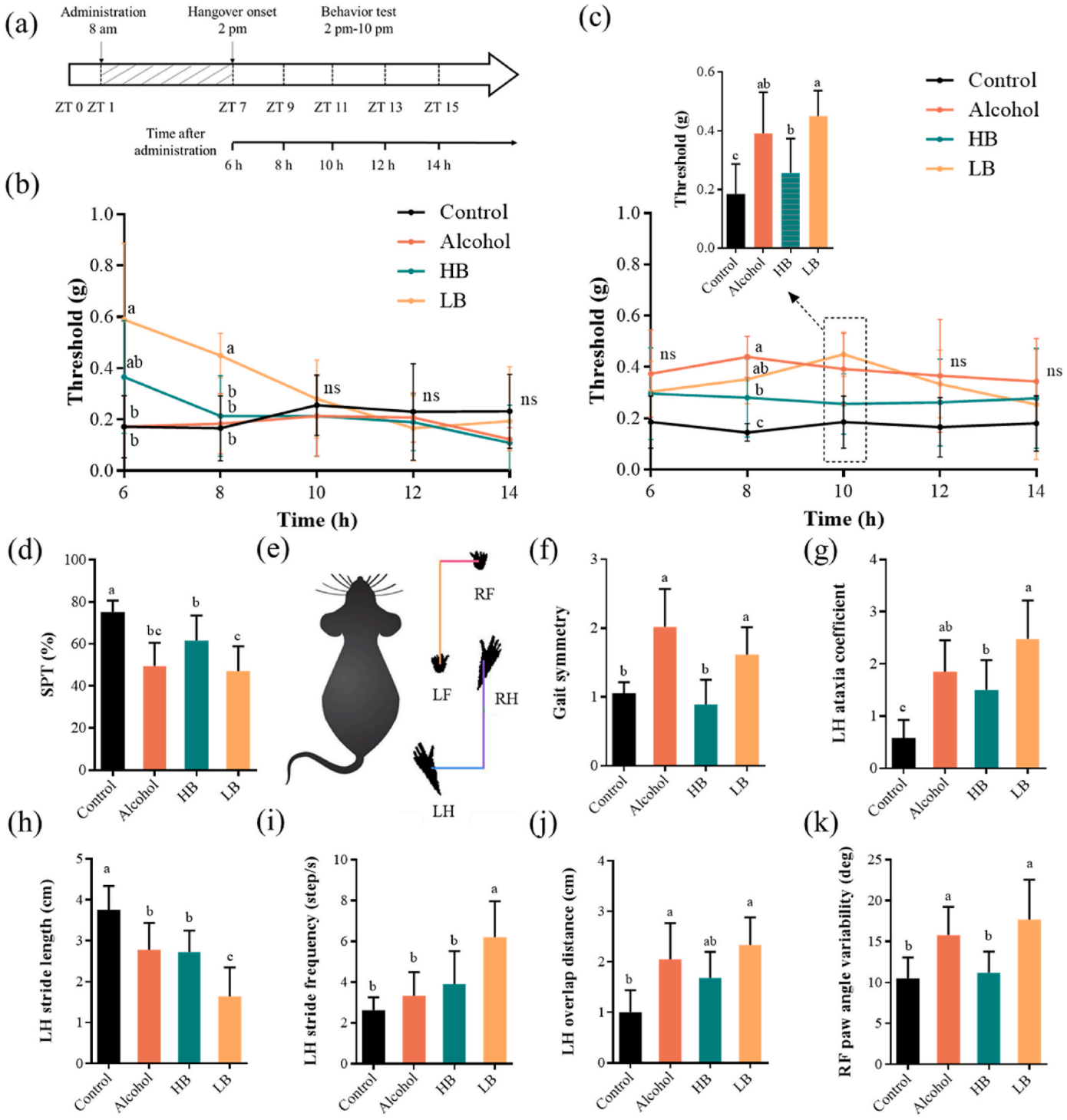

Fig. 3. Behavioural indices of mice after gavage. (a) Procedure of animal behavioural experiment, withdrawal thresholds of (b) periorbital and (c) pelma region, (d) sucrose preference of mice, (e) illustration for front and hind limb gait performance tested by CatWalk XT system, (f) gait symmetry, (g) LH ataxia coefficient, (h) LH stride length, (i) LH stride frequency, (j) LH overlap distance and (k) RF paw angle variability. ZT: Zeitgeber time, RF: right front limb, LF: left front limb, RH: right hind limb, LH: left hind limb. Different lowercase letters (a, b, c) represent significant ($p < 0.05$) differences between different groups of mice at the same time, and ns represent no significant differences between all the four groups.

time course from 6 to 14 h ($p > 0.05$).

In terms of other neurobiochemical parameters, such as serum ADH and brain histamine, no obvious differences were found between the HB and LB groups during the hangover state (Fig. S2).

3.4. Behavioral indices of hangover mice

Mouse behavioral indices, including pain thresholds, sucrose preference, and gait performance, were assessed to evaluate the hangover state (Fig. 3).

3.4.1. Mechanical pain threshold

Withdrawal thresholds were measured in the pelma and periorbital region of mice at 2-h intervals, starting from 6 h after gavage (Fig. 3a). Significantly high periorbital withdrawal thresholds were observed in the LB group at 6 h (0.59 g) and 8 h (0.45 g) after gavage ($p < 0.05$), which were approximately three times higher than those in the alcohol and control groups (Fig. 3b). For periorbital withdrawal thresholds of the other three groups, there were no significant differences observed throughout the experiment ($p > 0.05$). As for pelma withdrawal threshold, both the alcohol and LB groups showed a similar trend of first increasing and then decreasing, with the maximum thresholds of 0.44 and 0.45 g observed after gavage for 8 and 10 h, respectively (Fig. 3c). The pelma withdrawal thresholds of HB group were significantly ($p < 0.05$) lower than that of the LB group at 8 h (0.28 *vs*. 0.35 g) and 10 h (0.26 *vs*. 0.45 g). Nevertheless, significant higher pelma withdrawal thresholds were also observed in the HB group than the control group at 8 and 10 h ($p < 0.05$).

3.4.2. SPT

The anhedonia-like behavior of mice was analyzed through SPT to evaluate the hangover state (Fig. 3d). Oral administration of alcoholic samples significantly ($p < 0.05$) reduced the sucrose preference of mice in the alcohol, HB, and LB groups (all less than 61.50%) compared with the control group (75.20%). Nevertheless, mice in the HB group showed a significantly ($p < 0.05$) higher preference than those in the LB group (61.50% *vs.* 41.17%).

3.4.3. Gait performance

The gait performance of the hangover mice at 10 h after gavage was tested to evaluate the effects of different Baijiu samples on motor function using the CatWalk gait analysis system. The footprints of the four groups of mice were automatically recorded and various gait parameters were analyzed (Fig. 3e–k). After the administration of alcohol, the mice exhibited significantly ($p < 0.05$) increased gait symmetry (2.02 *vs.* 1.06), LH ataxia coefficient (1.86 *vs.* 0.58), LH overlap distance (2.05 *vs.* 1.00 cm), and RF paw angle variability (15.80 *vs.* 10.54°) in comparison with normal control. Additionally, the LH stride length in the alcohol group was significantly ($p < 0.05$) lower than that in the control group, indicating a walking deficiency caused by alcohol gavage. After gavage with HB sample, mice displayed slightly altered gait performance at 10 h compared to the control group. Two parameters were affected, including an increased LH ataxia coefficient (1.51 *vs.* 0.58) and a decreased LH stride length (2.72 *vs.* 3.76 cm). No evident differences were observed in the LH stride frequency or other indices between the two groups. Compared with those in the alcohol group, mice in HB group showed significantly ($p < 0.05$) lower gait symmetry and RF paw angle variability (0.89 *vs.* 2.02 and 11.19 *vs.* 15.80°, respectively), suggesting the gait performance was better in mice administered with the HB sample.

Regarding the LB group, impaired gait performance was detected compared with the other three groups at 10 h after administration. Specifically, the LH ataxia coefficient and LH overlap distance of LB group were about four times (2.48 *vs.* 0.58) and two times (2.33 *vs.* 1.00 cm) higher than those of the control group, respectively. The LH stride length (1.64 *vs.* 3.76 cm) was only half that of the normal control, and significantly increased gait symmetry and RF paw angle variability were observed in the LB group ($p < 0.05$). Moreover, mice in the LB group showed a significantly higher LH stride frequency than those in the control, alcohol, and HB groups ($p < 0.05$); while no evident differences were observed among these three groups. The results of other gait performance indices, such as LH swing speed and LH paw area, are shown in Fig. S3, as no significant differences were observed between the HB and LB groups ($p > 0.05$). Overall, CatWalk analysis revealed a remarkable walking deficiency in mice after gavage LB sample for 10 h, and several gait parameters of the HB group were affected by alcoholic sample administration. However, the impairment of gait performance was mild in the HB group compared with the LB group.

3.5. Correlation analysis between various parameters of mice and hangover symptom scores of populations

A correlation analysis between various parameters of mice with significant differences and hangover symptom scores of populations was conducted to investigate the potential of mice for characterizing the hangover effects following Baijiu consumption. As shown in Fig. 4a, two mouse indices, namely 10 h serum endothelin and 10 h LH ataxia coefficient, exhibited a profoundly positive correlation with all 24 hangover symptoms. The 10 h brain dopamine level and 10 h pelma withdrawal thresholds also demonstrated an intensely positive correlation with 22 out of 24 hangover symptoms. Additionally, remarkably positive correlations were found between hangover symptom scores and some gait-related indices, including RF paw angle variability, gait symmetry and LH overlap distance. Obviously positive correlations were also observed between mouse indices at other time points and hangover symptoms, such as the 8 h pelma withdrawal threshold and the 12 h serum endothelin. In contrast, negative correlations were found between some mouse indices and hangover symptoms. For example, sucrose preference, 8 h brain 5-HT, 10 h brain 5-HT, and 10 h LH stride length exhibited intensely negative correlations with hangover symptoms, including headache, reduced alertness, and depression. Notably, mice neurobiochemical and behavioral indices at 10 h, including serum endothelin, serum histamine, brain 5-HT, brain dopamine, pelma withdrawal threshold, gait symmetry, LH ataxia coefficient, LH overlap distance, LH stride length, LH stride frequency, RF paw angle variability, and sucrose preference, remarkably discriminate Baijiu samples of different post-drinking qualities in the OPLS-DA model (Fig. 4b).

3.6. Analysis of potential substances causing Baijiu hangover

The main compounds of Baijiu samples were tested, and a discrimination analysis was performed to investigate key components that may contribute to the differences in hangover severity. The total alcohol and aldehyde concentrations of the HB sample were significantly ($p < 0.05$) lower than those of LB sample (Fig. 5a). Furthermore, despite similar total ester and acid concentrations in both Baijiu samples, obvious differences were observed in specific compound compositions (Table S1). Then, the differentiation of Baijiu samples with different compositions as well as the variable importance in projection (VIP) values of main compounds were further investigated through an OPLS-DA model. As shown in Fig. 5b, the OPLS-DA model clearly distinguished the Baijiu samples. Data with VIP values greater than 1 are listed in Fig. 5c–and a total of 33 characteristic compounds that significantly contributed to the hangover severity were identified, including 14 esters, 7 alcohols, 5 aldehydes, 4 acids and 3 ketones.

A Spearman correlation analysis was performed to further reveal the relationship between neurobiochemical and behavioral indices of hangover mice and 33 key components of Baijiu screened out via OPLS-DA, and the result was provided in Fig. 6. Aldehydes in Baijiu, such as octanal, hexanal and 5-hydroxymethylfurfural, exhibited intensely positive correlations with serum endothelin, brain dopamine and a cluster of behavioral indices of mice, including RF paw angle variability,

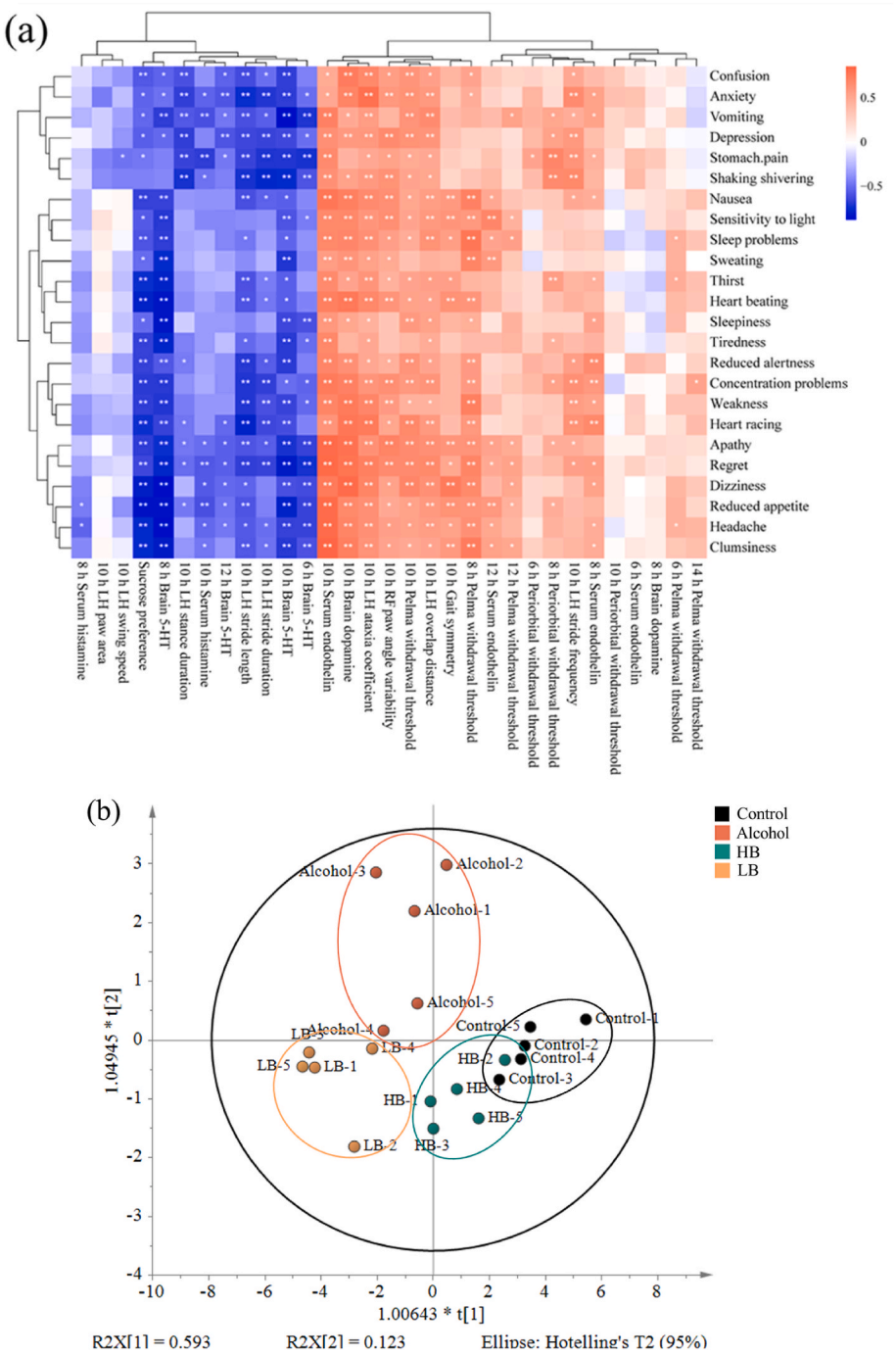

Fig. 4. OPLS-DA correlation analysis between the indices of mice and Baijiu hangover scores of populations. (a) Correlation heat map, (b) score plot. Positive and negative correlations are reflected by the color scale of red and blue, respectively. *$p < 0.05$ and **$p < 0.01$ according to Spearman's rank correlation coefficient. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

gait symmetry and pelma withdrawal threshold. Consistently, markedly positive correlations were observed for alcohols and these neurobiochemical and behavioral parameters, indicating triggering effect in the progress of hangover. Conversely, the predominant composition of esters, including ethyl lactate, ethyl acetate and ethyl isovalerate, displayed remarkably negative correlations with serum endothelin, brain dopamine, pelma withdrawal threshold and gait disorder-related parameters, suggesting attenuating effects during hangover stage.

4. Discussion

The hangover effects of different Baijiu samples were evaluated in a population following binge consumption, and a mouse model was established to characterize the hangover effects of Baijiu based on these samples. In current research, LB sample displayed obviously higher concentrations of total alcohols and aldehydes in comparison with HB sample, and remarkable differences were observed in the composition of esters and acids. This differences mainly depended on the yeast strain for brewing and may also affected by other factors such as raw materials, fermentation temperature, pH of mash (Cameleyre et al., 2015). The

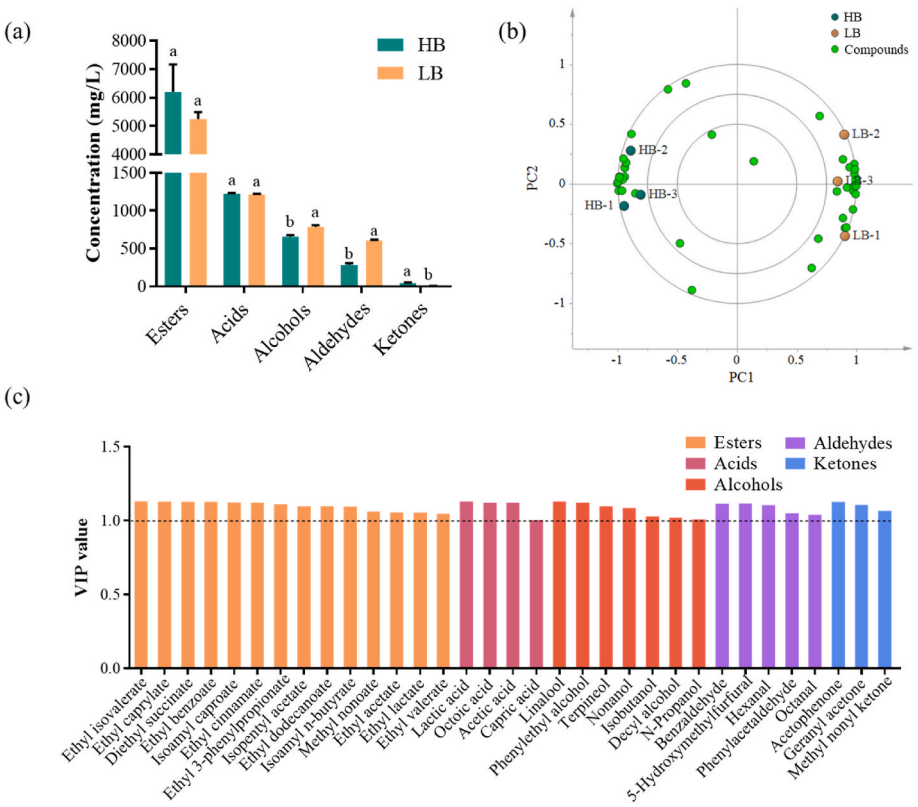

Fig. 5. Concentrations of different categories of main compounds in Baijiu samples and OPLS-DA of main compounds between Baijiu samples. (a) Concentrations of different categories of main compounds, (b) biplot and (c) VIP values of esters, acids, alcohols, aldehydes and ketones. Different lowercase letters (a, b, c) represent significant ($p < 0.05$) differences between different Baijiu samples.

variations in chemical constituents further determined the differences in post-drinking qualities of Baijiu samples. Despite the same alcohol content, LB sample resulted in generally more severe hangover symptoms after consumption, and higher hangover scores of headache, anhedonia-like behavior, and motor performance impairment were observed in populations administered with LB sample than alcohol and HB samples. Correlation analysis also indicated that alcohols and aldehydes were positively correlated with hangover severity, while esters seemed to attenuate hangover effect. Previous studies have shown that hangover severity is related to the concentration of the congeners in alcoholic beverages, and hangovers are more frequently experienced when consuming drinks with higher congener contents, such as brandy (Mackus et al., 2017; Rohsenow et al., 2010). Higher alcohols in alcoholic beverages are the main cause of accelerated intoxication, and excessive higher alcohols may lead to unexpected toxic effects on motor performance (Liu et al., 2021; Xie et al., 2018). Apart from those contained in the Baijiu samples, aldehydes can be generated by alcohol metabolism after Baijiu consumption. Increased aldehyde concentrations have been suggested to induce adverse effects, such as fatigue, headache, and dizziness (LoPachin & Gavin, 2014), which was consistent with our results. Other congeners, such as organic acids, have not been reported to be associated with hangover symptoms. However, they may cause unpleasant post-drinking experiences by affecting blood pH (Sun et al., 2020). The probable causes of alcohol hangovers are extensive and include various mechanisms such as dehydration, proinflammatory cytokine turbulence, and mitochondrial dysfunction; however, the fundamental etiology remains unclear (Köchling et al., 2019). Thus, the specific effects of each compound on hangover symptoms and its mechanisms should be further investigated.

The various hangover symptoms induced by consuming Baijiu were reflected by the alterations in the neurobiochemical parameters of mice. During hangover stage, a decrease in serum histamine was observed after administration of LB sample. In addition to alcohol consumption, the metabolism of alcohol's by-product, acetaldehyde, may lead to systemic histamine dysregulation (Merlo et al., 2017). It was also previously reported that low histamine level played a causative role in depression-like behaviors, impaired memory function, and abnormal sleep-wake cycles (Yamada et al., 2020). This was in accordance with the results of the hangover symptom evaluation obtained, including depression and clumsiness. Elevated serum endothelin concentrations were detected in hangover mice that were administered with alcohol and LB, and it showed strongly positive correlation with headache. Animal studies have demonstrated that endothelin potentially induced cortical spreading depression via an endothelin type A receptor-mediated mechanism, possibly vasoconstrictive ischemia (Vong et al., 2022). This 21-amino acid cyclic peptide has also been suggested to play a primary role in migraine attack induction (Iljazi et al., 2018), thus, differences in serum endothelin levels could be helpful in discriminating the headache-inducing effects of Baijiu samples. Additionally, the LB group showed lower brain 5-HT levels than the other groups. Similar situation has been reported by previous study, in which decreased brain 5-HT levels were found in mice administered with alcoholic beverage that contains high concentrations of congeners such as histamine (Zhao et al., 2022). The 5-HT plays a crucial role in the progression and modulation of pain, depression and anxiety, and decreased brain 5-HT levels have been observed in both migraine rats and depressed mice (Tian, Zou, et al., 2019; Zhang et al., 2017), which indicated that brain 5-HT level could characterize these symptoms. Reportedly, alcohol hangovers are at least partly characterized by neurobiochemical changes, including increased dopaminergic signaling. Elevated dopamine concentration was detected in brain tissue of hangover mice and positive correlations with headache and dizziness were observed. The increase in brain dopamine level may be attributed to the facilitating effects of alcohols and metabolites on dopaminergic

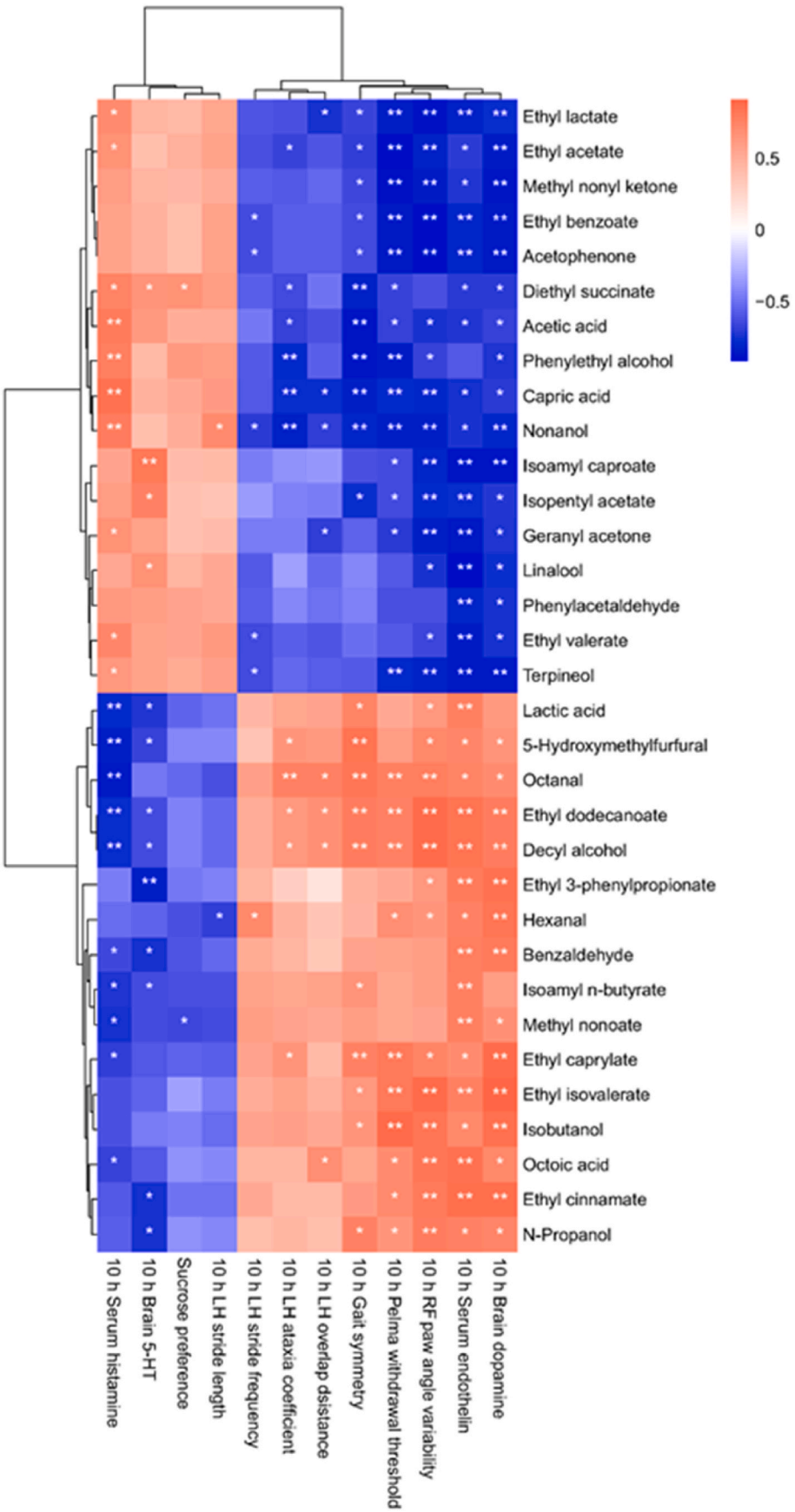

Fig. 6. Heatmap shows Spearman's rank correlations between the indices of mice and Baijiu components. Positive and negative correlations are reflected by the color scale of red and blue, respectively. *$p < 0.05$ and **$p < 0.01$ according to Spearman's rank correlation coefficient. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

signaling (Opitz et al., 2019). Moreover, population experiments have suggested that dopamine is involved in the pathology of migraine, and the administration of dopamine agonist has been shown to induce nausea, vomiting, and dizziness when compared with age-matched controls (Barbanti et al., 2020; Belkacemi & Darmani, 2020). Therefore, these mouse neurobiochemical parameters serve as suitable indices for characterizing headache- and depression-related symptoms during hangovers.

Besides the aforementioned neurobiochemical parameters, multi-parameter behavioral tests were also contributed to distinguish hangover states after Baijiu consumption. The withdrawal threshold has been commonly used to evaluate animal alertness, and an increased value in this measure has been typically observed in mice with reduced alertness (Huang et al., 2020). Similar result of mouse pelma withdrawal threshold was also observed in groups that were administered with alcoholic samples, particularly in the LB group, which may be ascribed to the intoxication effects of higher alcohols and other congeners (Sun et al., 2020). This result was in line with the scores of reduced alertness in population. Additionally, periorbital withdrawal threshold was used to assess pain intensity in mice with migraines (Zhang et al., 2017). Although some studies have suggested that hyperalgesia is a characteristic of hangovers in the population (Karadayian et al., 2013), a decrease in the periorbital withdrawal threshold was not observed in our study. The high thresholds observed in the LB group at 6 and 8 h may indicate that the mice were recovering from the intoxication stage. SPT is one of the most commonly used tests to evaluate depression-like behavior and anhedonia (Markov, 2022). Decrease in sucrose preference in mice was observed in our study, and it was intensely negative correlated to reduced appetite and depression. Similar results were reported in previous research when mice were administered with alcohol (Lee et al., 2017), which indicated that SPT is appropriate to characterize differences in anhedonia-like symptoms during hangover. Apart from the pain threshold and SPT, the neuromuscular coordination of hangover mice was evaluated based on gait performance. Impaired motor coordination was observed in all three alcoholic groups of mice during the hangover stage. Among them, the LB group exhibited the most severe ataxia, and strong correlations were found between gait performance parameters and motor-related symptoms such as dizziness. It has been suggested that a remarkable decline in stride length and velocity in mice may correspond to the gait deficits observed in patients with Parkinson's disease (Tatenhorst et al., 2016). Previous studies have also reported that alcohol administration leads to a decrease in both hindlimb and forelimb stride length in mice following the onset of alcohol hangovers, as well as loss of gait stability and walking deficiency (Karadayian & Cutrera, 2013). The results suggested these behavioral tests can characterize differences in hangover symptoms involving anhedonia-like behaviors and motor performance, such as reduced alertness, reduced appetite, dizziness and clumsiness.

5. Conclusion

Baijiu sample with higher concentrations of alcohols and aldehydes resulted in more severe hangovers in population and mice. Correlation analysis between mouse indices and population hangover scores further revealed that altered neurobiochemical parameters and impaired behavioral performance of mice were profoundly correlated with clinical hangover symptoms, such as headache, anhedonia-like feeling, and impaired motor performance. Moreover, remarkable correlations were observed between alcohols and aldehydes and these mouse indicators, including decreased brain 5-hydroxytryptamine, sucrose preference, increased serum endothelin and gait ataxia. These findings indicated that evaluating aforementioned indices in a BALB/c mouse model 10 h after administration can discriminate hangover effects of Baijiu samples with different post-drinking qualities under the same gavage dose, and Baijiu congeners may be responsible for variations in these mouse indices. Although more Baijiu samples should be included to further verify this model, this work may provide a perspective for identifying specific compounds that contribute to hangovers in alcoholic beverages.

CRediT authorship contribution statement

Yufei Liu: Writing – original draft, Visualization, Software, Investigation. **Qingxi Ren:** Writing – review & editing, Methodology. **Zhilei Zhou:** Resources, Methodology, Data curation. **Zhongwei Ji:** Methodology, Data curation. **Dongliang Ren:** Methodology, Data curation. **Yi Yang:** Methodology, Data curation. **Jian Mao:** Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2024.103799.

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Further-reading

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