



Research Article

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Antidepressant effects of *Radix bupleuri*–*Radix paeoniae* Alba: modulation of gut microbiota (*Lactiplantibacillus*/*Anaerotignum*) and restoration of phosphatidylcholine homeostasis along the gut–brain axis

Yaru CUI^{1*}, Minghui HU^{1*}, Chao LIU^{3,4}, Jialing XU¹, Yuxin TIAN¹, Shimeng LV¹, Zifa LI^{1,2}, Lidan WU^{1,2}, Hao ZHANG^{1,2}, Xinyu WANG^{1,2}, Sheng WEI^{1,2}

¹Institute for Chinese Medicine and Brain Science, Shandong University of Traditional Chinese Medicine, Ji'nan 250355, China

²Key Laboratory of Traditional Chinese Medicine Classical Theory, Ministry of Education/Shandong Key Laboratory of Innovation and Application Research in Basic Theory of Traditional Chinese Medicine/Shandong Provincial Engineering Research Center for the Prevention and Treatment of Major Brain Diseases with Traditional Chinese Medicine, Shandong University of Traditional Chinese Medicine, Ji'nan 250355, China

³Department of Traditional Chinese Medicine, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Ji'nan 250021, China

⁴First Clinical Medical College, Shandong University of Traditional Chinese Medicine, Ji'nan 250355, China

Abstract: Background: The pathogenesis of depression is associated with the gut–brain axis, involving gut microbiota and their metabolites. The herb pair *Radix bupleuri*–*Radix paeoniae* Alba (BP) has been reported to exert antidepressant effects by ameliorating gut microbiota dysbiosis but; its mechanisms within the gut–brain axis remain incompletely understood. Objectives and Methods: A chronic restraint stress (CRS) mouse model of depression was used. Following determination of the optimal BP dosage, mice were allocated into four groups: Control, Model, BP-treated, and fluoxetine-treated. Antidepressant effects were evaluated through behavioural tests. Serum metabolites were profiled using untargeted metabolomics and pathway enrichment analysis. Gut microbiota composition was assessed via 16S ribosomal ribonucleic acid (rRNA) gene sequencing, with analysis of alpha and beta diversity, and linear discriminant analysis effect size (LEfSe). Multi-omics integration was applied to investigate correlations between metabolites and microbial communities. Key metabolites in the hippocampus, colon, and cecum were quantified using enzyme-linked immunosorbent assay (ELISA). Results: BP treatment was associated with improvements in CRS-induced depressive-like behaviours. Metabolomic analysis indicated systemic metabolic disruption, with glycerophospholipid metabolism identified as the most significantly altered pathway. BP treatment was associated with changes in phosphatidylcholine (20:4/20:5) [PC(20:4/20:5)] and with restoration of CRS-related gut microbiota eubiosis, including changes in microbial diversity and composition. Integrated analysis revealed correlations between reshaped microbiota (such as increased *Lactiplantibacillus* and decreased *Anaerotignum*) and alterations in glycerophospholipid metabolism. Tissue measurements also showed a BP-associated pattern of lower phosphatidylcholine in the hippocampus and higher phosphatidylcholine in the colon. Conclusions: BP treatment was associated with ameliorated depressive-like behaviours, altered serum glycerophospholipid metabolism, normalisation of PC(20:4/20:5), restoration of gut microbial ecology, and tissue-specific differences in phosphatidylcholine levels along the microbiota–gut–brain axis. The observed PC redistribution was associated with

✉ Sheng WEI, weisheng@sdutcm.edu.cn
Xinyu WANG, wangxinyu0601@163.com
Hao ZHANG, zhanghaojune@126.com

* The two authors contributed equally to this work

Sheng Wei, <https://orcid.org/0000-0002-1326-4625>
 Xinyu Wang, <https://orcid.org/0000-0001-8612-2954>
 Hao Zhang, <https://orcid.org/0000-0001-6171-6174>

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antidepressant-like effects. These findings generate testable hypotheses; causal relationships remain to be validated in further intervention experiments.

Key words: Radix Bupleuri-Radix Paeoniae Alba herb pair; Depression; Metabolomics; Gut-brain axis; 16S rRNA sequencing; Non-targeted metabolomics

1 Introduction

Depression, a prevalent mood disorder characterised by persistently low mood, is associated with high rates of suicidality and disability (Matcham et al., 2023; Herzog et al., 2025). Its core clinical manifestations include depressed mood and anhedonia (Malhi and Mann, 2018). According to 2023 data from the World Health Organization, about 350 million individuals globally suffer from depression (Yu et al., 2024) and it is projected to become the leading cause of global disease burden by 2030. The pathogenesis of depression is complex and multifaceted. Recent research has shown that gut microbiota play a pivotal role in depression via the gut–brain axis (Dinan and Cryan, 2017). Acting as a key regulator within this axis, gut microbiota modulate cognitive function and mood through neuroendocrine and immune pathways (Foster and Mcvey Neufeld, 2013). Dysbiosis can impair the intestinal barrier, trigger the release of pro-inflammatory cytokines and alter short-chain fatty acid metabolism. Central nervous system (CNS) function is modulated via the vagus nerve, the hypothalamic–pituitary–adrenal (HPA) axis, and neurotransmitter synthesis pathways (e.g. serotonin), thereby inducing neuroinflammation and impairing synaptic plasticity (Cryan et al., 2020). Microbiota-targeted interventions, such as probiotics and faecal microbiota transplantation, offer novel therapeutic strategies for depression (Liu et al., 2015; Morais et al., 2021). Studies reveal that patients with depression exhibit reduced gut microbiota diversity, compromised intestinal barrier function, and significant disruptions in energy, lipid, and amino acid metabolism (Radford-Smith and Anthony, 2023; Zhou et al., 2023). Zheng et al. (Zheng et al., 2024) demonstrated that melatonin exerts antidepressant effects by modulating gut microbiota to regulate tryptophan–serotonin metabolism via the gut–brain axis. Additionally, Qian et al. (Qian et al., 2024) identified that 3-indolelactic acid, produced by *Bifidobacterium* strains, alleviates neuroinflammation through aryl hydrocarbon receptor activation, thereby conferring antidepressant effects. These findings indicate that gut microbiota exert antidepressant effects by modulating key metabolic pathways via the gut–brain axis, thereby influencing CNS function. Gut microbiota and their metabolites serve as critical mediators in the interplay among the nervous, endocrine, and immune systems regulating emotional brain function. Consequently, targeting the microbial community has emerged as an effective therapeutic strategy for depression.

Given the limitations of first-line antidepressants, such as selective serotonin reuptake inhibitors (SSRIs) and monoamine oxidase inhibitors (MAOIs)—including adverse effects and poor patient adherence—there is an urgent need to develop improved therapeutic strategies (Lin et al., 1995; Stone et al., 2009; Spellman and Liston, 2020). Accumulating evidence indicates that traditional Chinese medicine (TCM) exhibits multi-component, multi-pathway, and multi-target characteristics, demonstrating significant antidepressant efficacy and a favourable safety profile in clinical practice. Consequently, TCM has become a focal point in current depression treatment research. Among TCM formulations, the herb pair comprising *Radix bupleuri* and *Radix paeoniae* Alba (BP) is the most frequently used combination in antidepressant TCM prescriptions, and has shown significant antidepressant effects in both clinical trials and animal studies (Wang et al., 2016a; Wang et al., 2016b). Studies indicate that *Radix bupleuri* possesses anti-apoptotic, anti-inflammatory, antipyretic, sedative, and hepatoprotective properties (Jiang et al., 2020), while *Radix paeoniae* Alba exhibits anti-inflammatory, immunomodulatory, and hepatoprotective effects (Wang et al., 2020; Gu et al., 2024). The BP combination synergistically enhances hepatoprotective, analgesic, and anti-inflammatory effects, and ameliorates depressive-like behaviours in rats by restoring hippocampal damage through the modulation of amino acid metabolism and reduction of oxidative stress (Cai et al., 2025). Lv et al. (Lv et al., 2024) demonstrated that BP exerts antidepressant effects by increasing the abundance of *Lactobacillus* and

modulating tryptophan metabolism. Furthermore, Zhao et al. (Zhao et al., 2024) revealed that homovanillic acid, a metabolite of *Bifidobacterium longum*, attenuates depressive behaviours by inhibiting autophagic cell death and protecting impaired hippocampal neurons. Currently, the mechanisms underlying the antidepressant effects of BP are believed to involve mainly its related metabolites, which are thought to either directly regulate the central nervous system or modulate gut microbiota abundance. However, the extent to which these effects are mediated through the pivotal “gut–brain axis” pathway remains to be fully elucidated.

To systematically identify microbial and metabolic features associated with the antidepressant-like effects of BP extract, we used an integrated multi-omics strategy in a chronic stress-induced mouse model. The optimal therapeutic dose was first determined through comprehensive behavioural phenotyping. Subsequently, untargeted metabolomics and 16S rRNA gene sequencing were performed to profile systemic metabolic alterations and gut microbiota composition, respectively. Integrative bioinformatics analysis was then applied to delineate correlations between specific bacterial taxa and differential metabolites. This approach was designed to examine the hypothesis that BP-associated behavioural improvement is linked to modulation of the microbiota–gut–brain axis, a key mechanism underlying the antidepressant effects of this TCM herb pair. From a systems biology perspective, this study identifies a candidate microbiota–metabolite framework that may inform future therapeutic strategies for depression.

2. Materials

2.1 Animals

Male specific pathogen-free (SPF) C57BL/6J mice (7 weeks old, body weight 20 ± 2 g) were obtained from Vital River Laboratory Animal Technology Co., Ltd., Beijing, China; (licence number SCXKJing 2021-0006). All mice were housed in the Laboratory Animal Center of Shandong University of Traditional Chinese Medicine (licence number SYXKLu 2022-0009) under standard conditions. After one week of acclimatisation, mice were ear-tagged for identification and randomly assigned to groups (five mice per cage) with ad libitum access to food and water. Housing conditions were maintained at $23 \pm 2^\circ\text{C}$ and $55 \pm 10\%$ relative humidity, under a 12/12-h reversed light–dark cycle (lights on from 20:00 to 08:00). Daily handling was performed to minimise experimenter-induced stress. Experimental procedures conducted during the dark phase were performed under dim red illumination (<25 lux). All procedures were approved by the Institutional Animal Care and Use Committee of Shandong University of Traditional Chinese Medicine (Approval No. SDUTCM20230403002).

2.2 Chemicals and Reagents

Slices of *Radix bupleuri* and *Radix paeoniae* Alba were provided by the Huqiao Pharmaceutical Co., Ltd. (Bozhou, China). Fluoxetine hydrochloride dispersible tablets (FLX) were supplied by Eli Lilly Suzhou Pharmaceutical Co., Ltd. (Suzhou, China). Phosphate-buffered saline (PBS) was obtained from the Beijing Solarbio Science & Technology Co., Ltd., Beijing, China. Methanol, acetonitrile and formic acid were purchased from Fisher Scientific (USA). A magnetic bead-based faecal genomic DNA extraction kit was acquired from BioTeke Corporation (Wuxi, China). The mouse phosphatidylcholine (PC) quantification kit was obtained from Jianglai Biotechnology Co., Ltd. (Shanghai, China).

2.3 Drug Preparation and Phytochemical Characterisation

Radix bupleuri and *Radix paeoniae* Alba (600 g each) were coarsely ground into 0.5-cm pieces and extracted twice with 10 volumes of ethanol–water mixture (70% v/v) under reflux for 1.5 h per extraction. The combined extracts were allowed to settle and then filtered. The filtrate was concentrated to near-dryness by rotary evaporation at 60°C , followed by vacuum drying and gravimetric quantification of the resulting powder. The powder was stored at 4°C and reconstituted in appropriate solvents prior to use. The extraction yield of BP

extract was 16.4% (w/w).

For sample preparation, 0.1 g of BP extract was accurately weighed, dissolved in 10 mL of 70% methanol/water (1:100, w/v), sonicated at 45 °C for 5 min, and filtered through a 0.22-μm membrane for subsequent analysis.

Liquid chromatography (LC) analyses were performed using a Waters UPLC ACQUITY system equipped with an HSS T3 column (100 × 2.1 mm, 1.8 μm). The mobile phase consisted of 0.1% formic acid in water (A) and acetonitrile (B). Gradient elution was applied as follows: 0–2 min, 3–8% B; 2–10 min, 8–16% B; 10–20 min, 16–45% B; 20–25 min, 45–70% B; 25–30 min, 70–100% B. The column temperature was maintained at 40 °C, with a flow rate of 0.4 mL/min and an injection volume of 2 μL. Detection wavelengths were set at 254 nm and 262 nm and the photodiode array (PDA) scan range was 200–400 nm.

3 Results

3.1 Standardised quality control of the BP extract

To ensure the quality of BP extract, each batch was analysed via LC-MS. Consistent chemical profiles across batches confirmed the standardisation of the extract. Figure S1 shows the characteristic absorption patterns of chemical constituents at various time points and wavelengths, providing a comprehensive characterisation of the chemical composition. Comparative analysis of mass spectra and literature data enabled the identification of galloylsucrose, gallic acid, 1-O-β-D-glucosyl-8-O-benzoylpaeoniflorin sulfate, oxypaeoniflorin, albiflorin, mudanpioside E, paeoniflorin, galloylpaeoniflorin, 1,2,3,4,6-pentagalloylglucose, mudanpioside D, paeoniflorigenone, mudanpioside B, saikosaponin C, saikosaponin A, 2"-O-acetylsaikosaponin D, 3"-O-acetylsaikosaponin D, 6"-O-acetylsaikosaponin D, and saikosaponin D (Table 1).

Table 1 Qualitative analysis of selected compounds in *Radix bupleuri* and *Radix paeoniae Alba* extract

No.	Compound name	Chemical formula	Molecular weight	[M-H] ⁻ (m/z)	[M+HCOOH-H] ⁻ (m/z)	[M+H] ⁺ (m/z)	MS/MS fragments	Botanical source
1	Galloyl sucrose	C ₁₉ H ₂₅ O ₉	397.15	396.1415	442.1469	--	169.0148;331.0679	Radix Paeoniae Alba
2	Gallic acid	C ₇ H ₆ O ₅	170.02	169.0143	--	--	107.0137;124.0165	Radix Paeoniae Alba
3	1-O-β-D-Glucosyl-8-O-benzoylpaeoniflorin sulfate	C ₁₆ H ₂₄ O ₉	360.14	359.1363	405.1421	--	179.0717;197.0822	Radix Paeoniae Alba
4	Oxypaeoniflorin	C ₂₃ H ₂₈ O ₁₂	496.22	495.1251	--	497.1369	137.0239;165.0555;350.0570	Radix Paeoniae Alba
5	Albiflorin	C ₂₃ H ₂₈ O ₁₁	480.16	479.1290	525.1603	481.1413	121.0291;283.0819;357.1182;368.1216	Radix Paeoniae Alba
6	Mudanpioside E	C ₂₄ H ₃₀ O ₁₃	526.17	525.1337	571.1420	--	167.0708;179.0721;315.1243	Radix Paeoniae Alba
7	Paeoniflorin	C ₂₃ H ₂₈ O ₁₁	480.16	479.1284	525.1340	--	121.0293;165.0555;327.1078;449.1443	Radix Paeoniae Alba
8	Galloylpaeoniflorin	C ₃₀ H ₃₂ O ₁₅	632.17	631.1258	--	--	169.0139;313.0532;465.1415	Radix Paeoniae Alba
9	1,2,3,4,6-Pentagalloylglucose	C ₄₁ H ₃₂ O ₂₆	940.68	939.1559	--	--	469.0231;601.0826;769.0857	Radix Paeoniae Alba
10	Mudanpioside D	C ₂₄ H ₃₀ O ₁₂	510.28	509.1374	--	--	145.1204;239.1936	Radix Paeoniae Alba
11	Paeoniflorin	C ₂₃ H ₂₆ O ₁₀	462.15	461.1155	507.1210	--	121.0297;177.0	Radix Paeo-

12	genin Mudanpi- oside B	C ₃₁ H ₃₄ O ₁₄	630.13	629.1452	--	--	555 121.0294;431.1 344;553.1707	niae Alba Radix Paeo- niae Alba
13	Saikosapo- nin C	C ₄₈ H ₇₈ O ₁₇	926.52	925.5143	971.5723	--	161.0449;617.4 040;779.4574	Radix Bu- pleuri
14	Saikosapo- nin A	C ₄₂ H ₆₈ O ₁₃	780.46	779.4572	825.4765	--	161.0453;617.4 050	Radix Bu- pleuri
15	2"-O-Acetyl- saikosaponin D	C ₄₄ H ₇₀ O ₁₄	822.47	821.4680	867.4975	--	127.0403;617.4 047;779.4571	Radix Bu- pleuri
16	3"-O-Acetyl- saikosaponin D	C ₄₄ H ₇₀ O ₁₄	822.47	821.4683	867.4970	--	617.4061;683.4 174;761.4474	Radix Bu- pleuri
17	6"-O-Acetyl- saikosaponin D	C ₄₄ H ₇₀ O ₁₄	822.47	821.4798	867.4958	--	617.5204;761.5 797;779.5923	Radix Bu- pleuri
18	Saikosapo- nin D	C ₄₂ H ₆₈ O ₁₃	780.47	--	825.4747	--	161.0449;279.2 308;617.4029	Radix Bu- pleuri

Note: "--" indicates that the corresponding ion was not detected under the experimental conditions. [M-H]⁻, [M+HCOOH-H]⁻, and [M+H]⁺ represent the deprotonated molecular ion, formate adduct ion, and protonated molecular ion, respectively. MS/MS, tandem mass spectrometry.

3.2 Antidepressant effects of BP in mice

To determine the optimal antidepressant dose of BP, mice were randomly assigned to five groups: control (Con, saline), fluoxetine (FLX, positive control, 18 mg/kg), low-dose BP (BPL, 1.312 g/kg), medium-dose BP (BPM, 2.624 g/kg), and high-dose BP (BPH, 5.248 g/kg). Following 14 days of oral administration, depressive-like behaviours were assessed using OFT, FST, and TST (Fig. 1a).

After two weeks of BP treatment, no significant differences in total distance travelled in the OFT were observed among groups (Figs. 1b and 1c), indicating that BP did not measurably alter locomotor activity under these conditions. Similarly, there were no significant differences in entries into the central zone among groups (Fig. 1d), suggesting that BP did not influence anxiety-like behaviours. In the FST, immobility time was significantly reduced in the BPM, BPH, and FLX groups compared with the control group, whereas no significant reduction was observed in the BPL group (Fig. 1e). Regarding FST immobility frequency, the BPH group showed a significant increase compared with the control group but no significant differences were found among the FLX, BPM, and control groups (Fig. 1f). In the TST, immobility frequency was significantly decreased in the BPM, BPH, and FLX groups compared with the control group, while the BPL group showed no significant decrease (Fig. 1g). Although immobility time in the TST did not differ significantly among groups, a decreasing trend was observed in the FLX, BPL, BPM, and BPH groups relative to the control group (Fig. 1h). The FUST and SST were used as validated behavioral paradigms specifically designed to assess anhedonia-like behaviors, serving as alternatives to the sucrose preference test (SPT) to avoid potential confounding factors associated with chronic gavage administration. Collectively, these behavioural observations were associated with antidepressant-like effects of BP, with the medium dose selected for subsequent experiments.

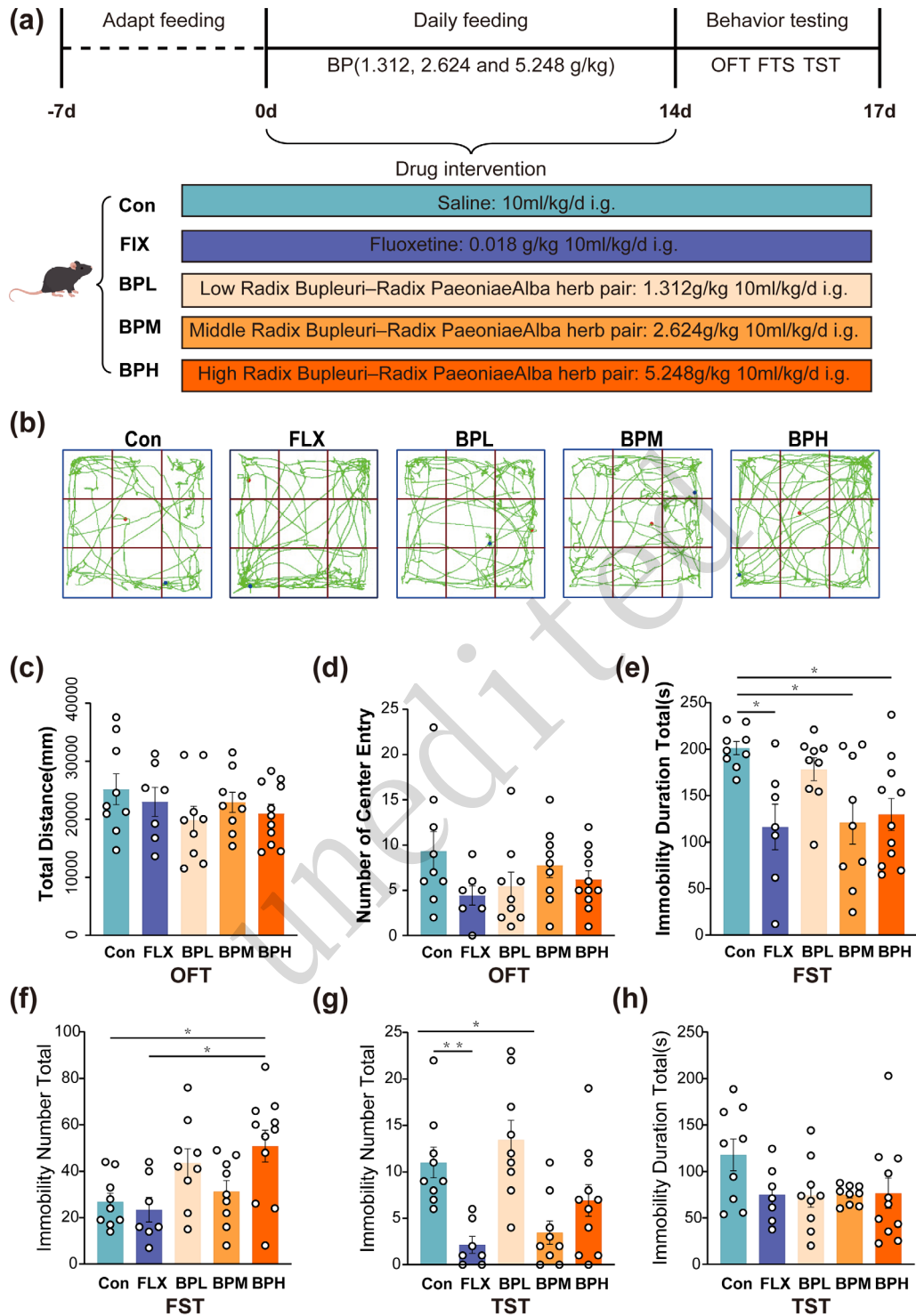


Fig. 1 Antidepressant effects of BP in mice. (a) Experimental timeline for efficacy evaluation. (b) Representative movement traces from the open field test (OFT). (c) Total distance travelled in the OFT (n = 7–11). (d) Number of entries into the central zone in the OFT (n = 7–11). (e) Immobility time in the forced swim test (FST; n = 7–11). (f) Number of immobility episodes in the FST (n = 7–11). (g) Number of immobility episodes in the tail suspension test (TST; n = 7–11). (h) Immobility time in the TST (n = 7–11). Data are presented as mean $\pm$ SEM. *: $P < 0.05$, **: $P < 0.01$.

3.3 Alleviation of depression-like behaviours by BPM in chronic restraint stress (CRS) -induced mice

To clarify the effects of BP on mice subjected to CRS, a therapeutic intervention study was conducted. Mouse models were established using the CRS paradigm, and medium-dose BP was administered to investigate its antidepressant efficacy. Animals were divided into four groups: control (Con), model (Mod), fluoxetine (FLX), and medium-dose BP (BP). All groups received intragastric administration for 14 days concurrent with CRS exposure. Subsequently, depressive-like behaviours were evaluated using the OFT, FUST, SUST, and TST (Fig. 2a).

After two weeks of treatment, no significant differences in total distance travelled in the OFT were observed among groups (Figs. 2b and 2c), indicating that neither fluoxetine nor BP measurably altered motor function. Second, in the FUST, sniffing duration was significantly reduced in the Mod group compared with the Con group but was significantly increased by both fluoxetine and BP treatment relative to the Mod group. The difference in sniffing duration between female urine and saline was also significantly diminished in the Mod group compared with the Con group, an abnormality that was also attenuated by fluoxetine and BP (Fig. 2d). Third, in the SUST, grooming latency was significantly increased in the Mod group compared with the Con group, whereas it was significantly decreased in both the BP and FLX groups (Fig. 2e). Concurrently, grooming time was significantly reduced in the Mod group compared with the Con group; this reduction was significantly reversed by fluoxetine and BP treatment (Fig. 2f). Finally, in the TST, both immobility duration and frequency were significantly increased in the Mod group compared with the Con group (Figs. 2g and 2h), and these measures were significantly lower in the BP- and fluoxetine-treated groups. Overall, BP treatment was associated with improvement across the measured CRS-induced depressive-like behavioural outcomes.

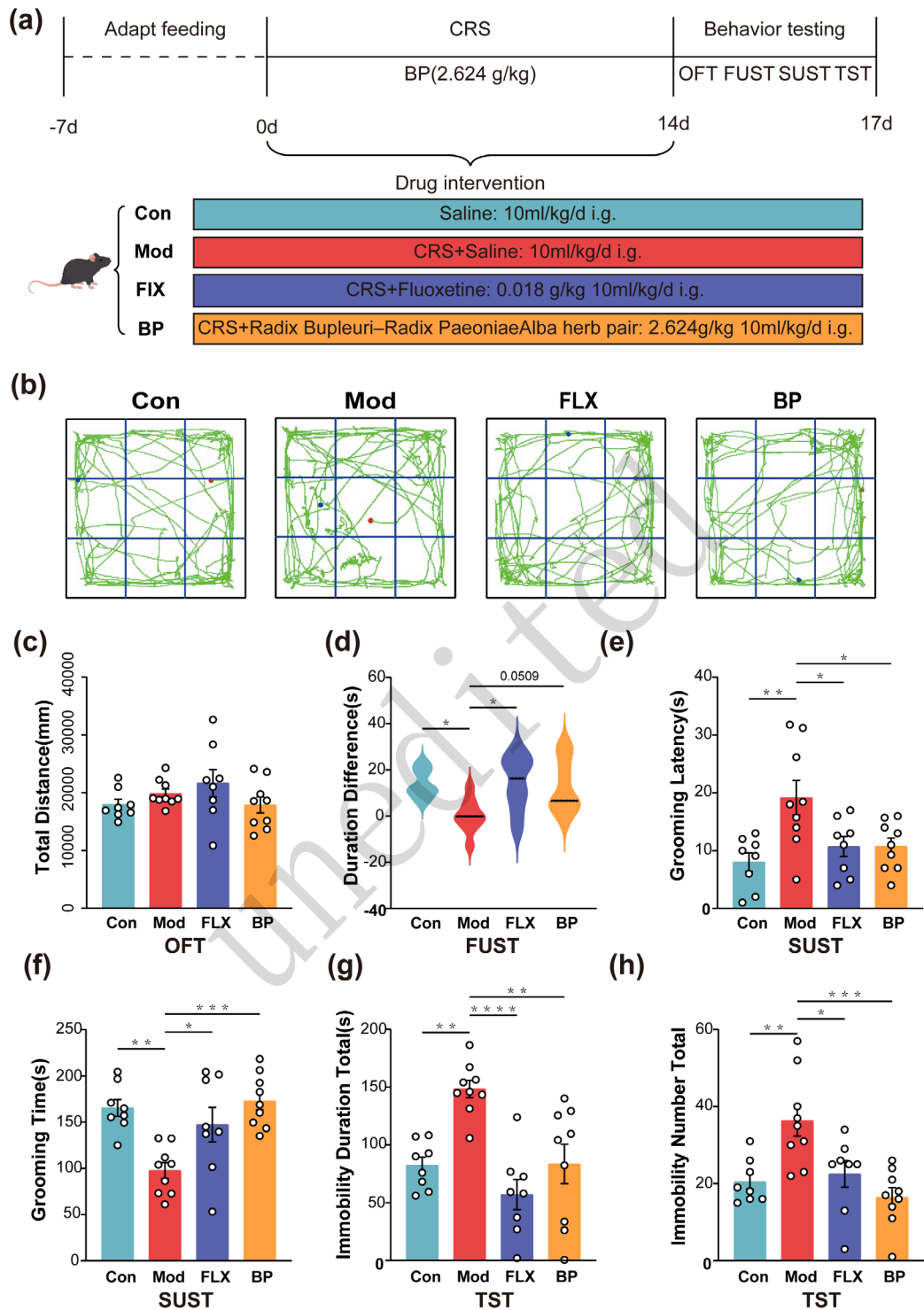


Fig. 2 Amelioration of depression-like behaviours by BP in mice subjected to CRS. (a) Schematic of the CRS model protocol. (b) Representative open field test (OFT) movement traces. (c) Total distance travelled in the OFT (n = 8–9). (d) Duration of sniffing in the female urine sniffing test (FUST; n = 8–9). (e) Grooming latency and (f) grooming duration in the sucrose spray test (SUST; n = 8–9). (g) Immobility time and (h) number of immobility episodes in the tail suspension test (TST) (n = 8–9). Data are presented as mean ± SEM. *: P < 0.05, **: P < 0.01, ***: P < 0.001.

3.4 Metabolic effects of BP in a mouse model of depression

Metabolomic studies have revealed altered metabolic profiles during the progression of depression. To investigate the antidepressant mechanisms of BP via metabolic regulation, untargeted serum metabolomics was performed to identify key metabolic targets. First, a systematic analysis of global metabolic alterations was conducted. Partial least squares-discriminant analysis (PLS-DA) revealed distinct clustering between the Con and Mod groups, as well as between the BP and Mod groups (Figs. S2a–2d). These results indicate significant metabolic differences among the three groups, confirming that both CRS and BP intervention alter metabolic composition. Subsequently, a detailed analysis of metabolic changes was performed. A total of 1,154 metabolites were identified (Fig. S3). Compared with the Con group, 109 metabolites were significantly altered in the Mod group (Fig. 3a). Relative to the Mod group, 62 metabolites were significantly changed in the BP-treated group (Fig. 4a). To elucidate the metabolic pathways underlying the antidepressant effects of BP, differential metabolites between the Con and Mod groups, and between the BP-treated and Mod groups, were mapped to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. KEGG enrichment analysis identified 20 significantly enriched pathways between the Con and Mod groups (Fig. 3b) and 21 altered pathways between the BP-treated and Mod groups (Fig. 4b). Notably, four metabolic pathways were co-regulated by both BP treatment and CRS: glycerophospholipid metabolism, central carbon metabolism in cancer, biosynthesis of plant secondary metabolites, and non-ribosomal peptide structures of siderophore groups. Moreover, glycerophospholipid metabolism was significantly enriched by differential metabolites across all three groups, suggesting its potential involvement in the antidepressant mechanisms of BP.

Given its prominence in the enrichment analysis, the glycerophospholipid metabolism pathway was selected for in-depth investigation. All metabolites within this pathway were further analysed (Fig. 5). A total of 97 metabolites were identified. Among these, PC(20:4(5Z,8Z,11Z,14Z)/20:5(5Z,8Z,11Z,14Z,17Z)) was the sole differential metabolite common to all three experimental groups. Its level was significantly lower in the Mod group than in the Con group, and significantly higher in the BP-treated group than in the Mod group. Furthermore, the associations of BP treatment with multiple PCs within this pathway were examined (Fig. 6 and Fig. S4). A decreasing trend was observed in the serum levels of several PCs following BP administration, including PC(18:1(9Z)/18:0), PC(18:2(9Z,12Z)/15:0), PC(18:2(9Z,12Z)/20:4(5Z,8Z,11Z,14Z)), and PC(20:2(11Z,14Z)/18:1(11Z)). These observations indicate an association between BP treatment, depressive-like behavioural improvement, and phosphatidylcholine metabolism but do not establish mediation.

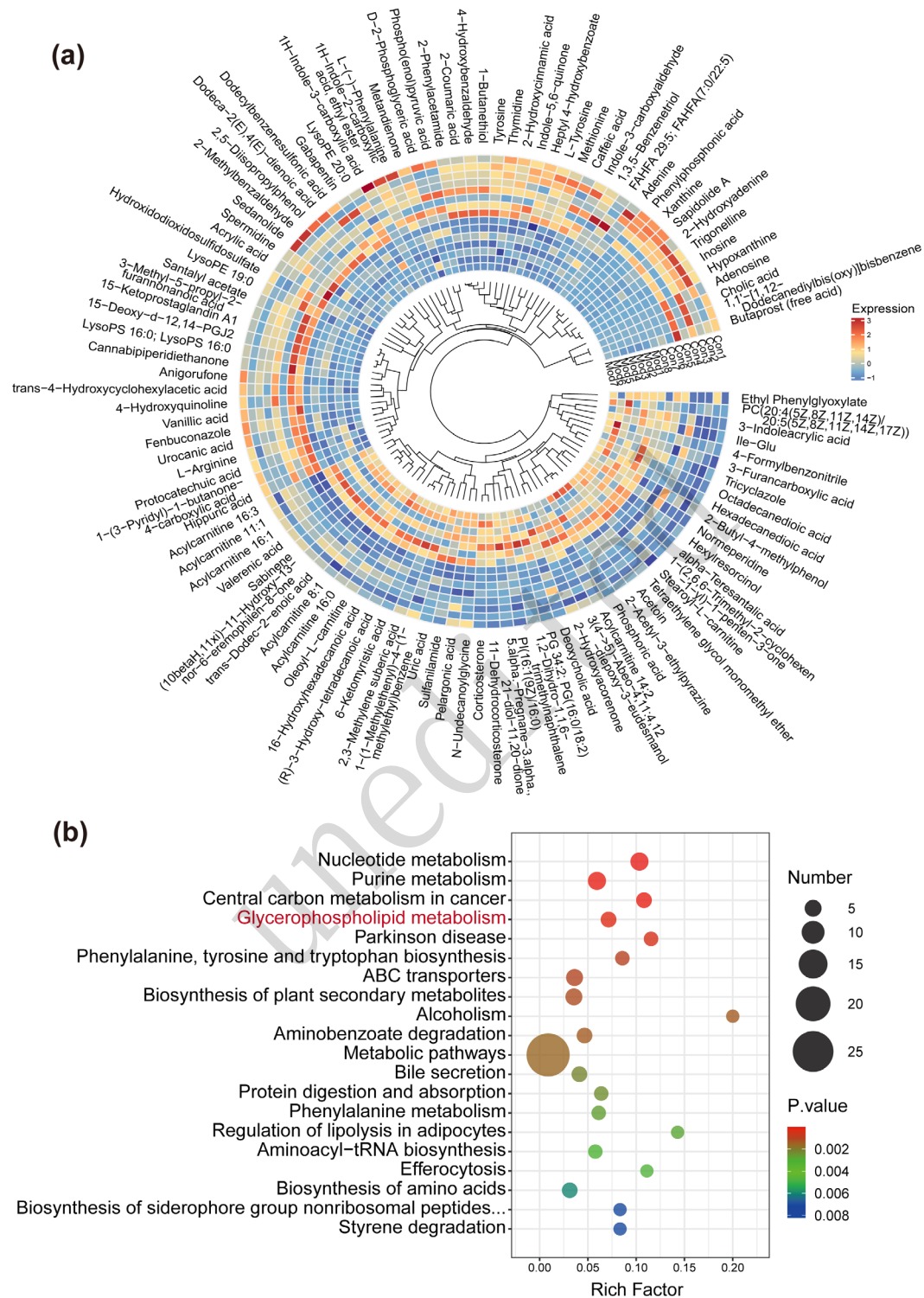


Fig. 3 Differential metabolites and metabolic pathways between Con and Mod groups. (a) Volcano plot of differentially abundant metabolites. (b) Enriched metabolic pathways identified between Con and Mod groups. Data are presented as mean $\pm$ SEM. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

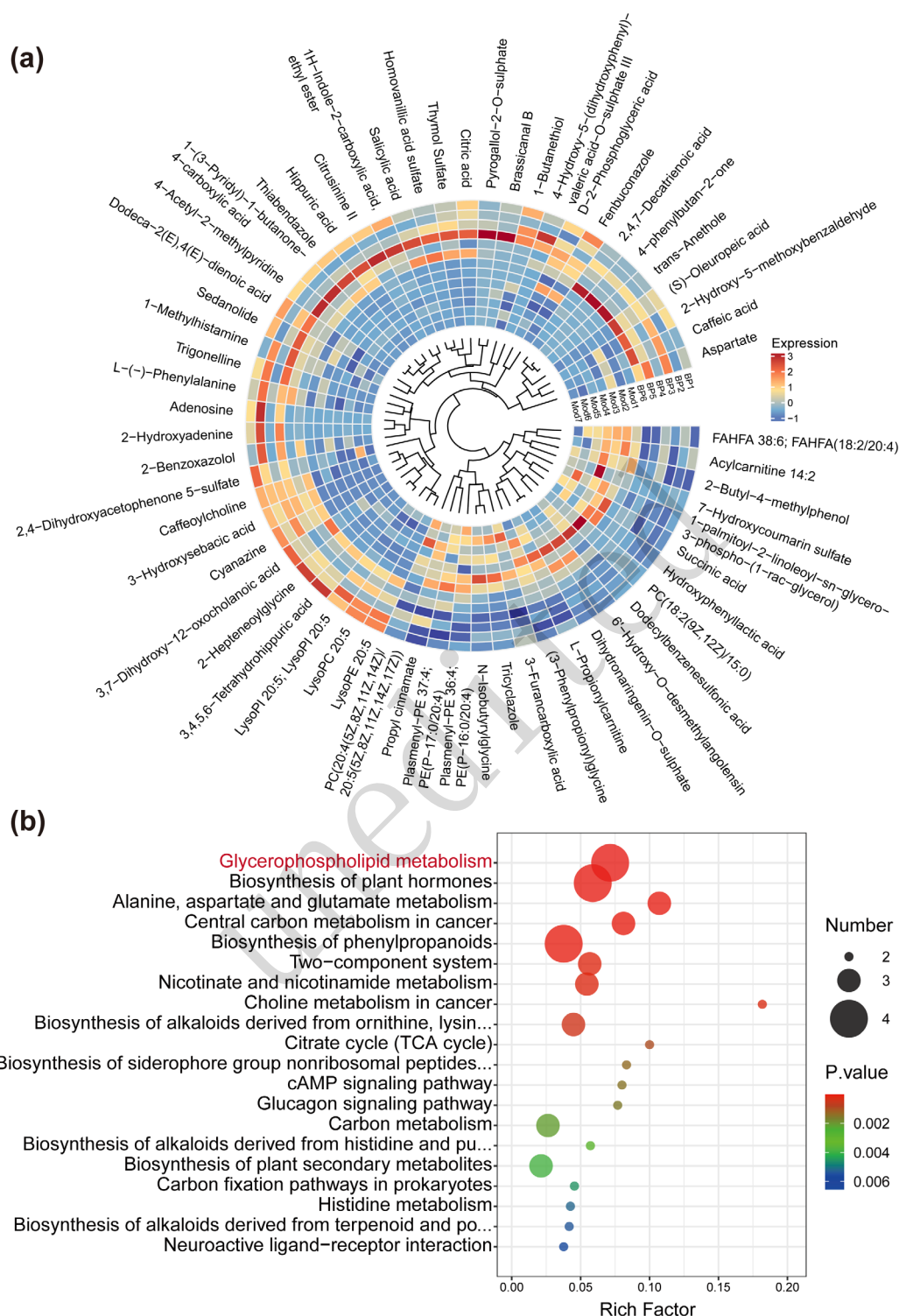


Fig. 4 Differential metabolites and metabolic pathways between BP-treated and Mod groups. (a) Volcano plot of differentially abundant metabolites. (b) Enriched metabolic pathways identified between BP and Mod groups. Data are presented as mean $\pm$ SEM. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

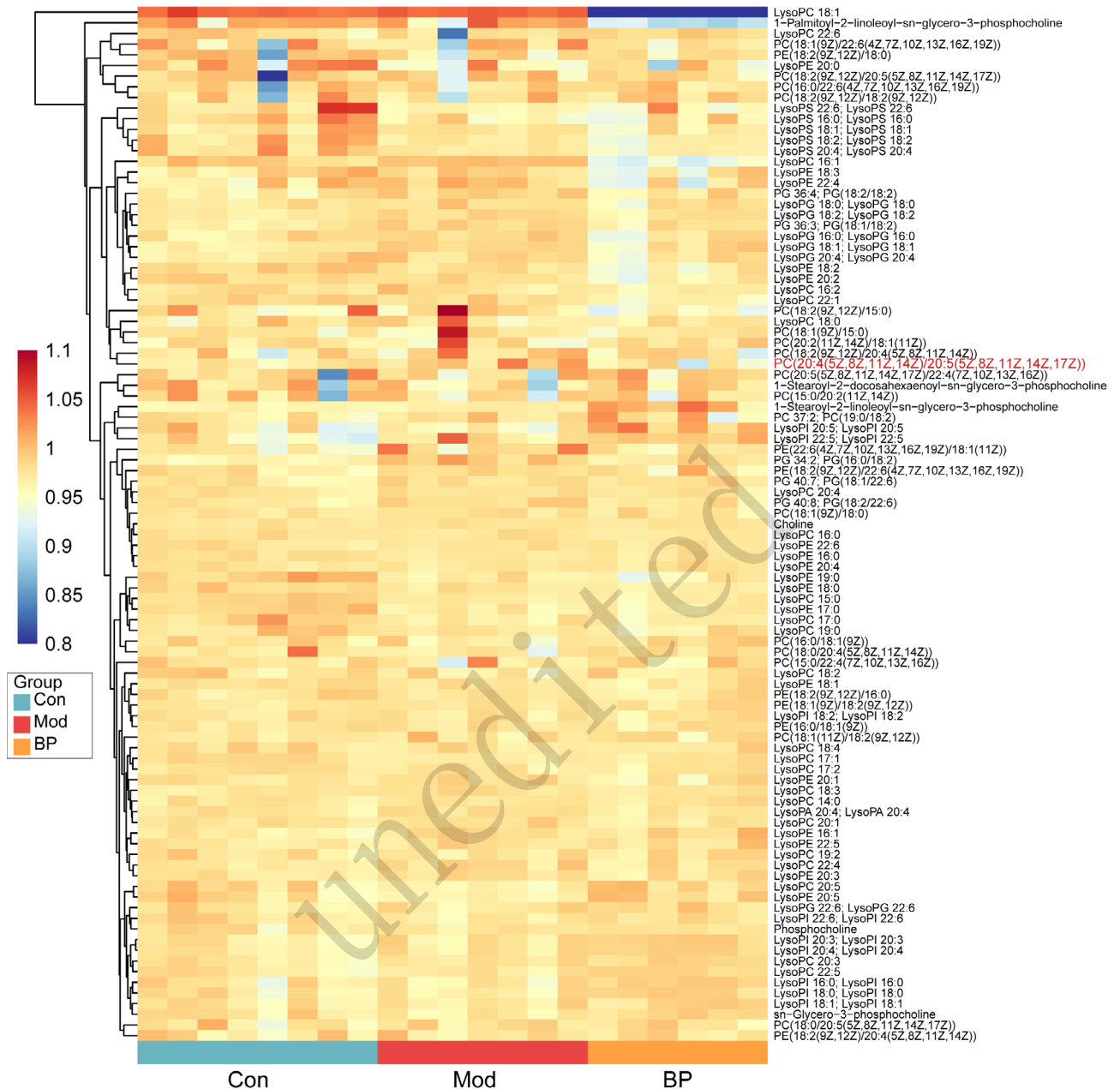


Fig. 5 Glycerophospholipid metabolite levels in the Con, Mod, and BP Groups. Colour intensity reflects relative abundance: dark red indicates higher levels, whereas dark blue indicates lower levels.

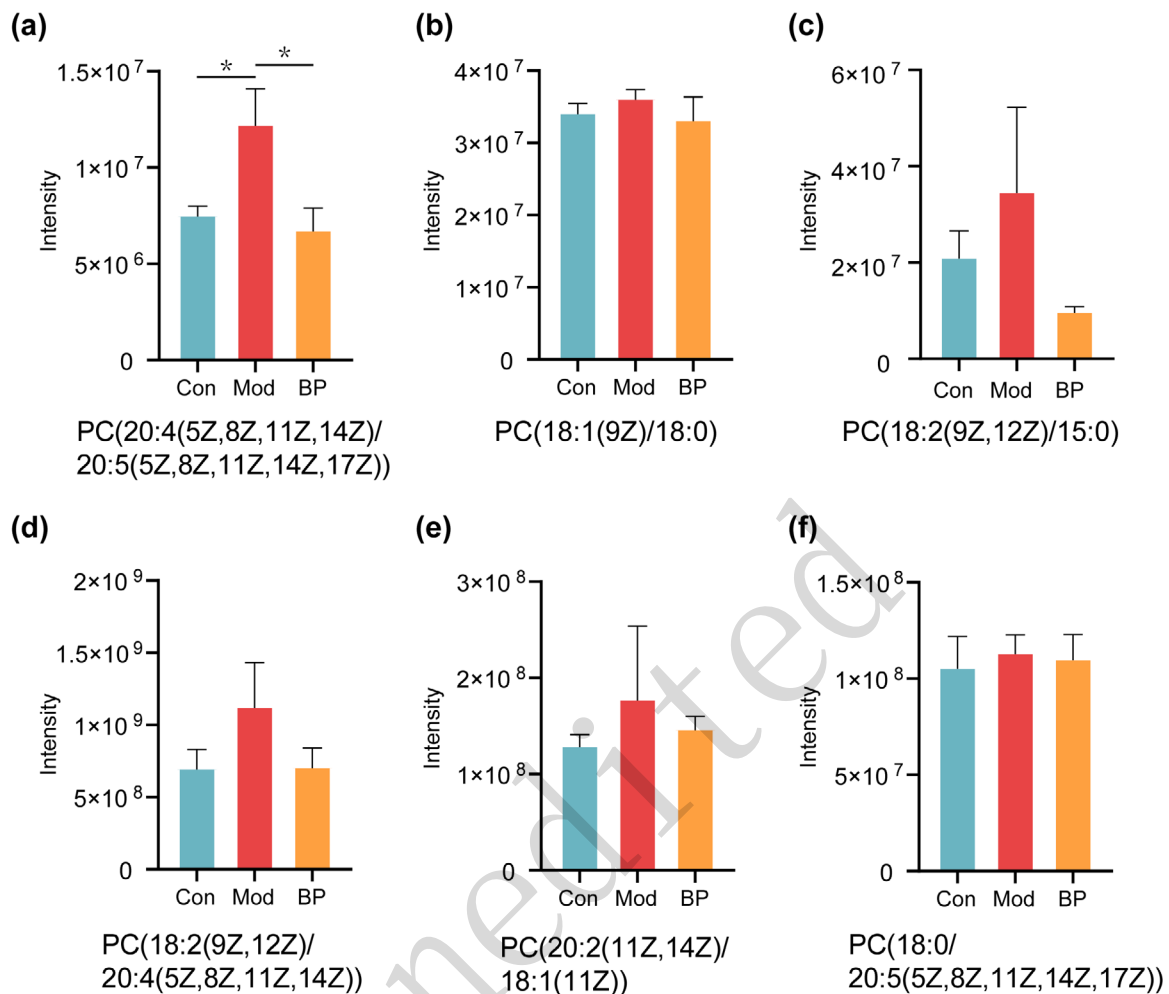


Fig. 6 Altered phosphatidylcholines in the glycerophospholipid metabolism pathway. Differential ions were identified using integrated univariate (fold change, Student's t-test) and multivariate (PLS-DA VIP score) criteria. An ion was deemed differential only if it satisfied all three conditions: fold change ≥ 1.5 or ≤ 0.67 , adjusted P-value < 0.05 (Benjamini-Hochberg correction), and VIP score ≥ 1 . Data are presented as mean $\pm$ SEM. *: $P < 0.05$.

3.5 Effects of BP on gut microbiota in a murine model of depression

The gut microbiota, a critical component of the intestinal barrier, is indispensable for maintaining homeostasis within the gut-brain axis. To assess the impact of BP on microbial richness and diversity in mice with CRS-induced depression, α -diversity indices and operational taxonomic unit (OTU) counts were evaluated. A total of 7,431 OTUs were identified across 21 samples from the Con, Mod, and BP-treated groups. Venn diagram analysis revealed 3,706 OTUs in the Con group, 3,137 in the Mod group, and 3,259 in the BP group. A core set of 806 OTUs was shared among all groups, with 1,178 overlapping between the Con and Mod groups, and 1,063 between the Mod and BP groups. Distinct OTU profiles were observed: 2,098 were unique to the Con group, 1,702 to the Mod group, and 1,766 to the BP group (Fig. 7a). Alpha diversity analysis indicated a significant reduction ($P < 0.05$) in the Observed index in the Mod group compared with the Con group, whereas BP intervention exhibited a restorative trend in depressed mice (Fig. 7b). No significant differences in Shannon indices were observed among the three groups (Fig. 7c). These data show that CRS induction reduced

α -diversity and OTU abundance in the murine gut microbiota, while BP administration restored microbial diversity and increased OTU counts. Furthermore, principal coordinates analysis (PCoA) based on the Bray–Curtis dissimilarity metric was performed to evaluate the effects of BP on β -diversity in CRS-induced mice (Fig. 7d). The results indicated a clear separation in gut microbiota composition between the Con and Mod groups. Following BP intervention, the microbiota composition of the BP group diverged from that of the Mod group and shifted towards the Con group profile. This suggests that CRS induced significant alterations in microbiota structure, which were ameliorated by BP treatment. In summary, CRS induced gut microbiota dysbiosis in mice, which was effectively remodelled by BP. Subsequent analysis examined alterations in gut microbiota across various taxonomic levels. At the phylum level, the relative abundances of *Firmicutes*, *Verrucomicrobiota*, and *Desulfobacterota* were significantly decreased in the Mod group compared with the Con group, whereas those of *Bacteroidota* and *Proteobacteria* were markedly increased. Compared with the Mod group, the BP group showed significantly increased relative abundances of *Firmicutes* and *Desulfobacterota*, alongside marked decreases in *Bacteroidota* and *Verrucomicrobiota* (Fig. 7e). Additionally, the *Firmicutes/Bacteroidota* ratio was significantly reduced in the Mod group relative to the Con group; this ratio was partially restored following BP treatment.

At the genus level (Fig. 7f), the relative abundances of *unclassified Clostridiales*, *Akkermansia*, and *unclassified Clostridia UCG-014* were significantly lower in the Mod group than in the Con group, whereas *unclassified Muribaculaceae*, *Lachnospiraceae NK4A136* group, and *Alistipes* exhibited higher abundances. Following BP treatment, *unclassified Clostridiales*, *unclassified Firmicutes*, and *Clostridium* were significantly enriched, while *unclassified Muribaculaceae* and *Lachnospiraceae NK4A136* group were reduced relative to the Mod group.

Finally, Linear Discriminant Analysis Effect Size (LEfSe) was conducted to identify predominant microbial taxa in each group (Fig. 8). The analysis identified *Akkermansia*, *unclassified Oscillospiraceae*, *Eisenbergiella*, *Oscillibacter*, *unclassified Clostridiales Family XIV Incertae Sedis*, *Dubosiella*, *Incertae Sedis*, *Roseburia*, and *UCG-005* as predominant taxa in the Con group. In the Mod group, predominant taxa included *Anaerotruncus*, *Anaerotignum*, *Paramuribaculum*, *Bilophila*, *Ligilactobacillus*, *unclassified Peptococcaceae*, *Parasutterella*, and *Christensenellaceae R-7* group. The BP group showed significant enrichment of *Clostridium*, *Lactobacillus*, *Oscillibacter*, *Desulfovibrio*, *HT002*, and *Lactiplantibacillus*. Further analysis focused on genus-level alterations across all experimental groups. The top 30 differentially abundant genera were identified between the Con and Mod groups, and a comparative analysis was performed between the BP and Mod groups (Figs. 9a and 9b). A total of 13 differentially abundant genera were common to all three groups (Fig. 9c). Specifically, the abundances of *Anaerotignum*, *Paramuribaculum*, and *Anaerotruncus* were significantly elevated in the Mod group compared with both the Con and BP groups. Conversely, the abundances of *Clostridium*, *Lactobacillus*, and *Lactiplantibacillus* were markedly reduced in the Mod group.

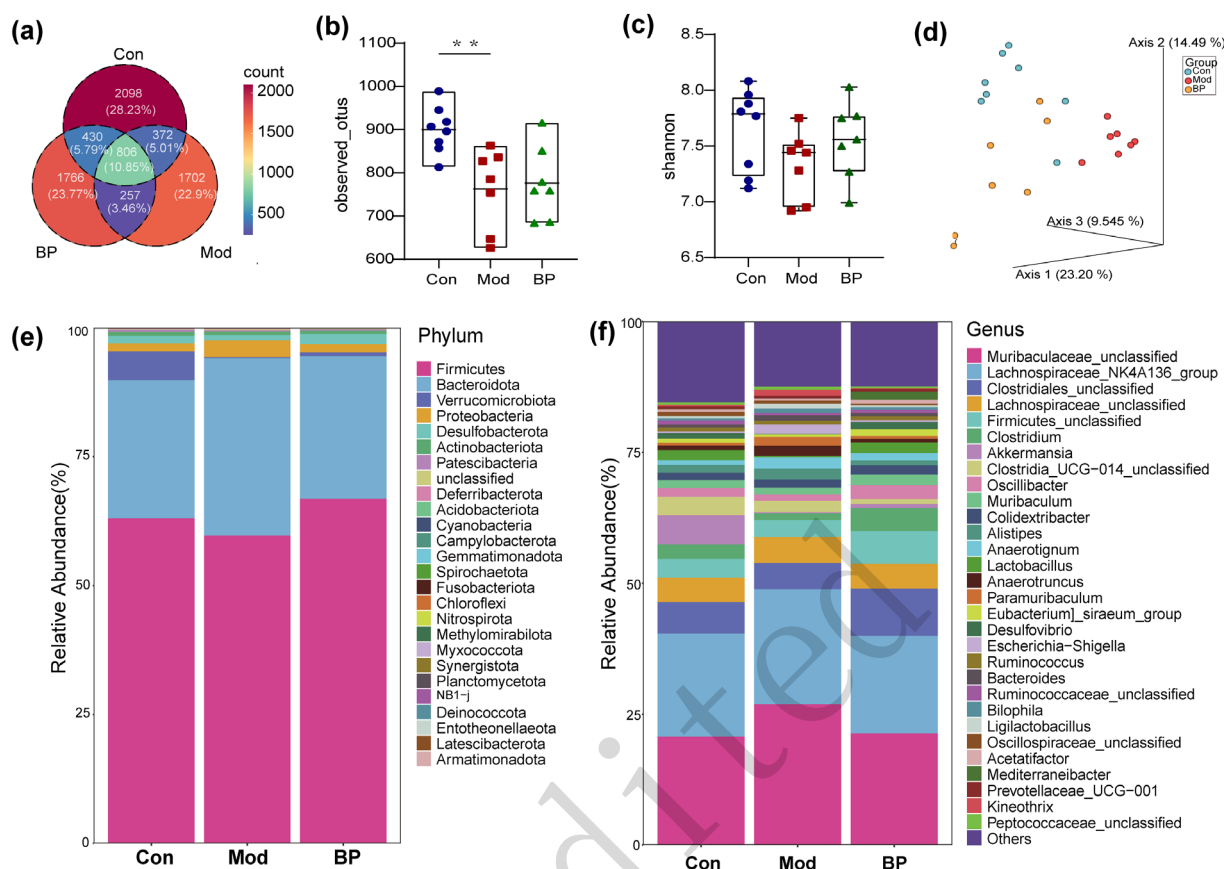


Fig. 7 Modulation of gut microbiota composition by BP in mice subjected to CRS. (a) Venn diagram illustrating shared and unique operational taxonomic units (OTUs). (b) Observed species index (richness). (c) Shannon index (richness and evenness). (d) Principal coordinates analysis (PCoA) based on Bray–Curtis dissimilarity. Relative abundance at the (e) phylum and (f) genus levels. Data are presented as mean $\pm$ SEM. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

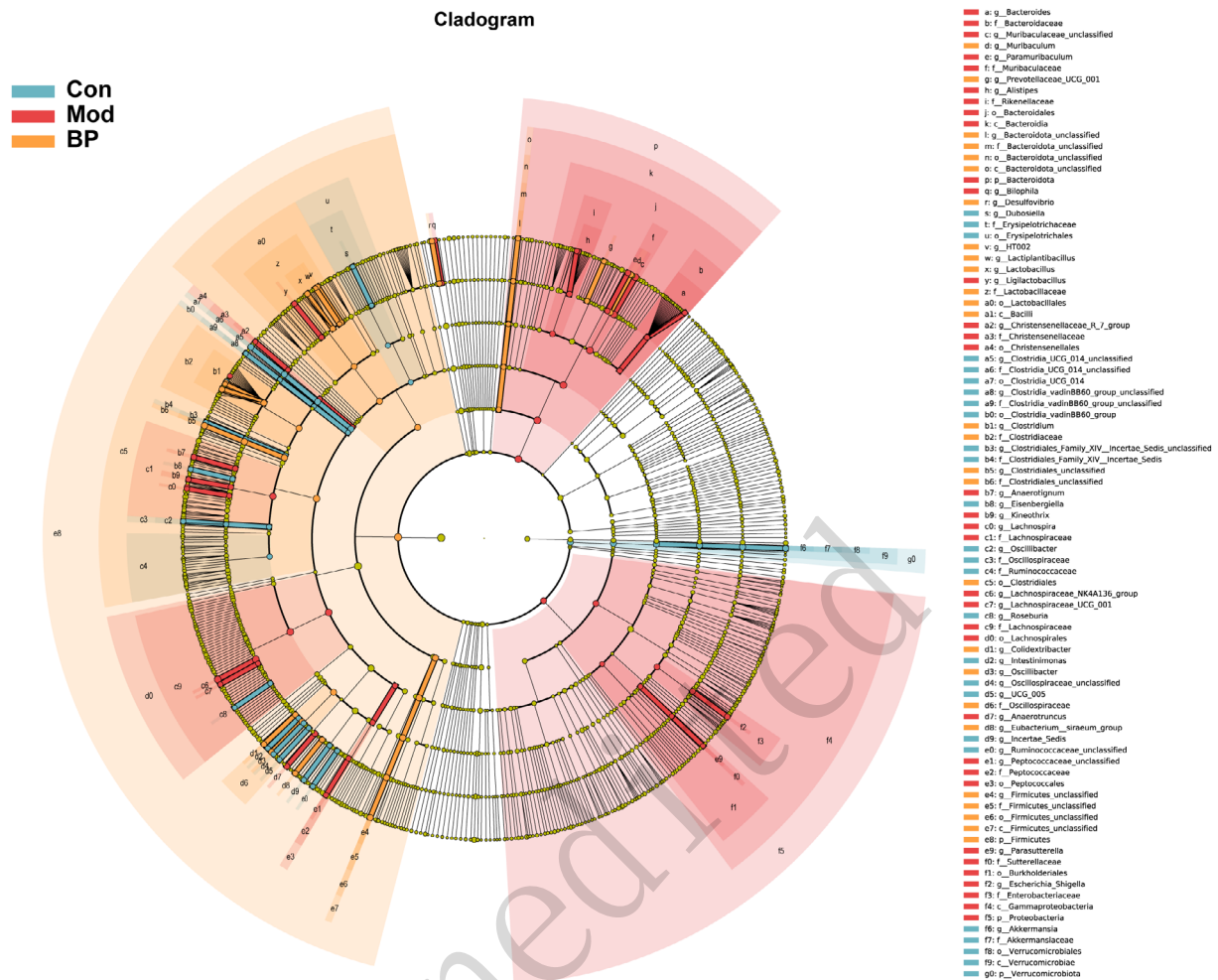


Fig. 8 Linear discriminant analysis (LDA) cladogram. The cladogram depicts taxonomic groups with significant differential abundance between experimental groups, as determined by LDA effect size (LEfSe) analysis.

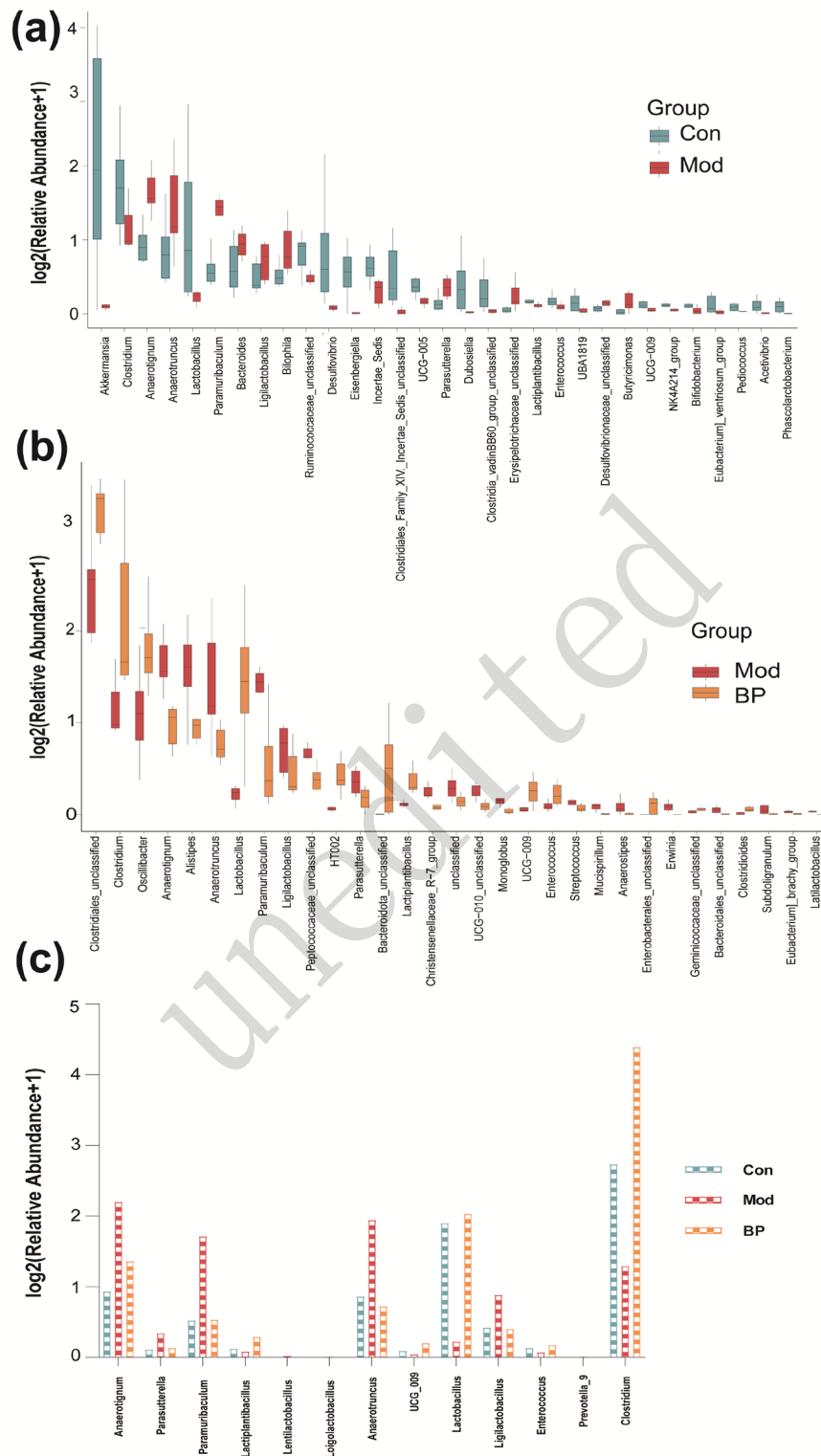


Fig. 9 Associations of *Lactiplantibacillus* and *Anaerotignum* with the antidepressant effects of BP. (a) Differential microbiota at the genus level between the Con and Mod groups. (b) Differential microbiota at the genus level between the BP and Mod groups. (c) Common differential microbiota at the genus level among the Con, Mod, and BP groups.

3.6 Correlation analysis

To elucidate the antidepressant mechanisms of BP via the microbiota–gut–brain axis, a multi-omics correlation analysis was conducted, focusing on key bacterial taxa associated with glycerophospholipid metabolism and potential PC transport to the brain. In the Con versus Mod comparison (Fig. 10a), several bacterial genera exhibited broad correlations with differential metabolites. Specifically, *Lactiplantibacillus*, *Anaerotignum*, *Lentilactobacillus*, and *Loigolactobacillus* correlated with 60–80% of the altered metabolites in the target pathway. A broader correlation spectrum (80–100%) was observed in the BP versus Mod comparison (Fig. 10b) for *Lactiplantibacillus*, *Lactobacillus*, *Anaerotruncus*, *Anaerotignum*, and *Lentilactobacillus*. A key finding was the exclusive and strong correlation of both *Lactiplantibacillus* (beneficial) and *Anaerotignum* (pathogenic) with the common metabolite PC. Thus, significant links were established between these two bacterial genera, glycerophospholipid metabolism, and PC levels.

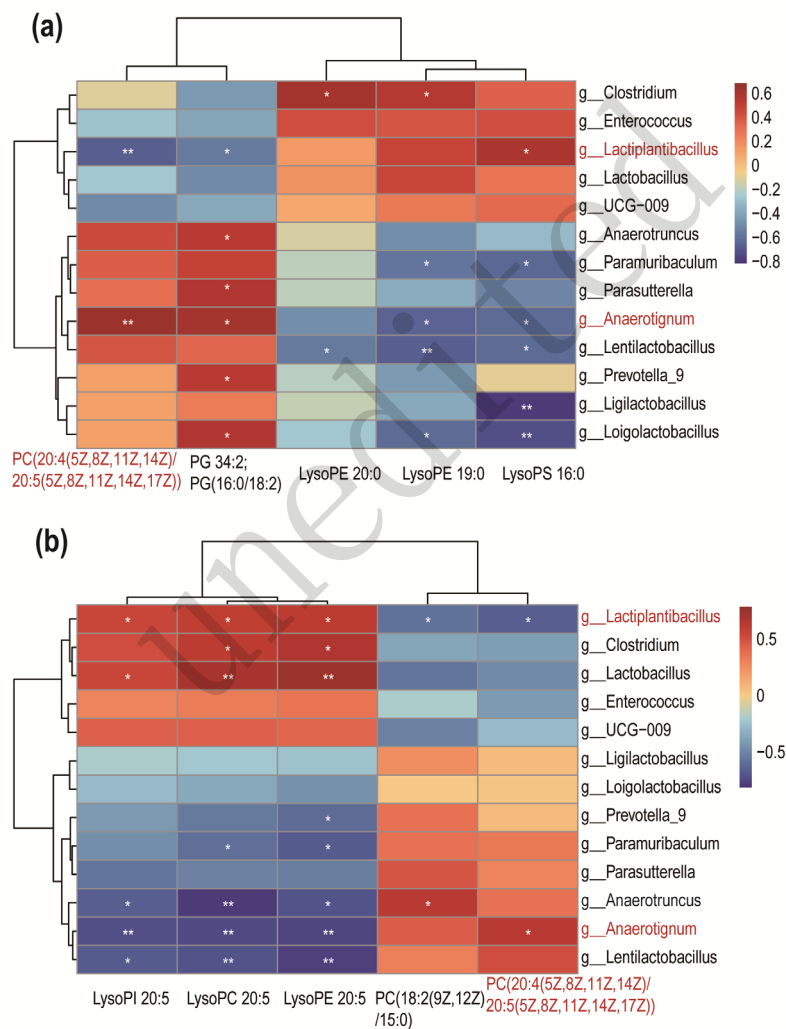


Fig. 10 Correlations between differential glycerophospholipid metabolites and altered bacterial genera. (a) Correlations between control (Con) and model (Mod) groups. (b) Correlations between Bupleurum–Paonia treated (BP) and Mod groups. Red and blue squares denote positive and negative correlations, respectively; colour intensity reflects correlation strength. Significant correlations are indicated as *: $P < 0.05$ and **: $P < 0.01$.

3.7 Modulation of peripheral and hippocampal metabolites by BP treatment in model mice

Recent studies have shown that polysaccharides from the TCM Tongxie Yaofang (TXYF) modulate the gut microbiota and restore bile acid metabolism via the microbiota–gut–brain axis, thereby alleviating depression-like behaviours induced by chronic unpredictable stress (Chen et al., 2023b). Consequently, we hypothesised that BP alleviates CRS-induced depression-like behaviours through the modulation of gut microbiota and PC. Based on this study, we propose that BP exerts antidepressant effects by regulating serum PC. To investigate whether BP affects peripheral and central PC levels in model mice, ELISA kits were used to measure the concentration of PC in tissue from the hippocampus and colon, and in the contents of the caecum (Figs. 11a–11c). The results revealed that hippocampal PC levels were significantly elevated in the Mod group compared with the Con group, whereas a significant decrease was observed in the BP group relative to the Mod group (Fig. 11a). In the colon, PC levels were significantly higher in both the Con and BP groups compared with the Mod group (Fig. 11b). No significant differences were detected among the three groups in the PC levels of the cecal contents (Fig. 11c). These tissue-specific observations are consistent with an association between PC distribution and antidepressant-like effects, but do not demonstrate transport between compartments or a causal mechanism.

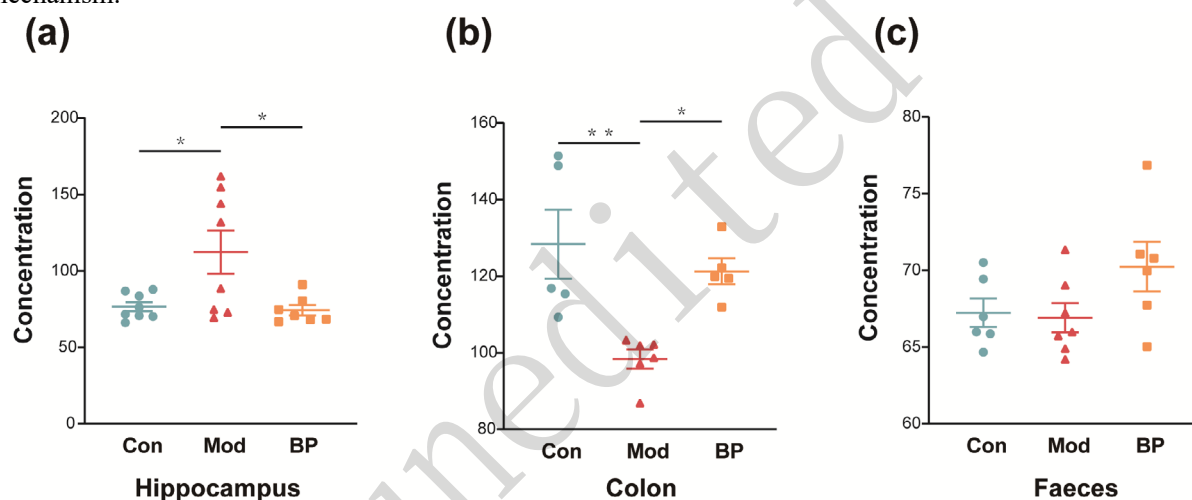


Fig. 11 PC concentrations in hippocampal tissue, colonic tissue, and caecal contents. (a) Hippocampal PC levels. (b) Colonic PC levels. (c) Cecal content PC levels. Data are presented as mean $\pm$ SEM. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

4 Discussion

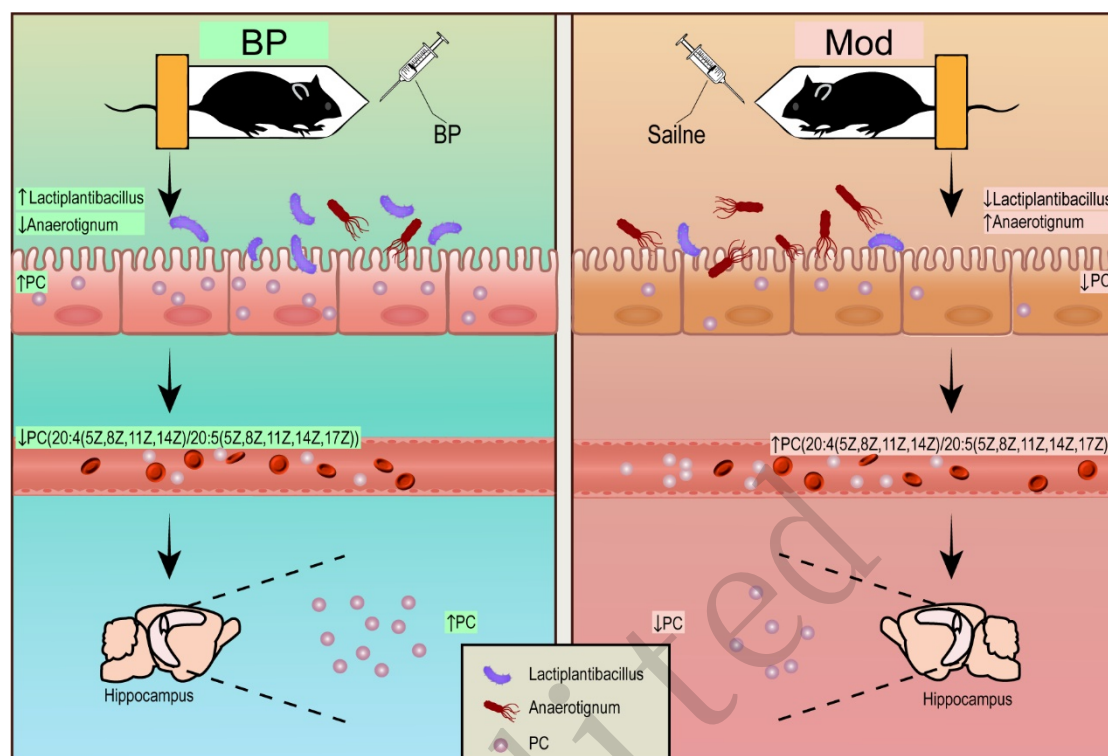


Fig. 12 Proposed associations of BP antidepressant effects with the gut–brain axis.

This study elucidated the potential antidepressant mechanisms of BP through an integrated approach combining metabolomics, gut microbiota sequencing, and multiple experimental techniques. BP treatment was accompanied by changes in gut bacterial genera, including increased *Lactiplantibacillus* and decreased *Anaerotignum*, as well as alterations in systemic glycerophospholipid metabolism. Serum, colonic, and hippocampal measurements showed a compartment-specific pattern of PC differences after BP treatment (Fig. 12). Collectively, these observations support an association among BP treatment, the microbiota–metabolite–gut–brain axis, and antidepressant-like behavioural outcomes but do not establish a causal pathway.

BP is widely used in the treatment of depression (Chen et al., 2022; Lv et al., 2022; Cai, et al., 2025), anxiety (Lin et al., 2025), and hepatitis (Huang et al., 2023). Previous work by Lv et al. (Lv, et al., 2022) suggested that the antidepressant effects of BP are mediated mainly through the alleviation of oxidative stress, regulation of the HPA axis, and neuronal protection. However, research regarding the synergistic interactions between the distinct targets and signalling pathways involved in BP's antidepressant mechanisms remains limited (Lv, et al., 2022). Li et al. (Li et al., 2021) observed that BP exhibited superior antidepressant effects compared with Bupleurum or Paeonia administered alone. Furthermore, Chen et al. (Chen et al., 2023a) reported that BP mediates antidepressant effects by regulating cis-aconitic acid decarboxylase and D-amino acid oxidase. Recent evidence links depression to altered gut microbiota diversity and functionality (Belkaid and Harrison, 2017), a finding consistent with our results. Patients with depression exhibit altered microbiota diversity and specific taxonomic abundances compared with healthy controls, establishing associations between dysbiosis and depression (Simpson et al., 2021). The phyla *Firmicutes*, *Actinobacteria*, and *Bacteroidetes* show the most significant alterations in depression (Zheng et al., 2016). Faecal microbiota transplantation (FMT) from depressed patients induces depression-like behaviours in rodents, suggesting that dysbiosis may precede and causally contribute to depression (Sittipo et al., 2019)). Species such as *Bacteroides thetaiotaomicron*, *B.*

fragilis, and *B. uniformis* have been identified as promoting depression (Guida et al., 2018). Conversely, the probiotic strain *L. plantarum* 299v alleviates major depression by modulating pro-inflammatory cytokines, cortisol, and the kynurenine pathway (Rudzki et al., 2019). These data indicate a strong correlation among gut microbiota, metabolism, and depression. Jayanama et al. (Jayanama et al., 2022) reported higher faecal *Anaerotignum* abundance in prediabetic versus normoglycaemic HIV-positive patients. We speculate that *Anaerotignum* may influence energy metabolism, although mechanistic insights remain limited. The present study combined serum metabolomics with 16S rRNA sequencing to examine associations among BP treatment, gut microbiota, glycerophospholipid metabolism, and PC levels in the colon, serum, and hippocampus. In our data, *Lactiplantibacillus*, *Anaerotignum*, and glycerophospholipid metabolism were among the features most strongly associated with the BP-treated phenotype. This reveals crucial microbiota–host metabolic crosstalk, providing novel insights into depression pathogenesis. However, the correlations observed here do not identify the direction of the effect among microbial abundance, PC metabolism, and behaviour.

PC is the most abundant phospholipid in living organisms and serves as a core structural component of all eukaryotic cell and organelle membranes (Fagone and Jackowski, 2013; Bellot et al., 2023). While the liver is the central organ for PC synthesis, dietary intake represents a crucial exogenous source (Li and Vance, 2008). Beyond influencing membrane structure, PC promotes lipid metabolism and acts as a precursor for signalling molecules such as arachidonic acid and acetylcholine, thereby participating in the regulation of inflammation and mood. PC deficiency directly compromises membrane structural integrity and may impair myelin formation and acetylcholine synthesis (Tavasoli et al., 2020; Kenny et al., 2023; Kozon-Markiewicz et al., 2023). Conversely, excess PC disrupts lipid metabolic homeostasis and increases the risk of cardiovascular diseases (Bellot, et al., 2023). Huang et al. demonstrated that dietary choline supplementation significantly increased total phospholipid and PC levels in the hippocampus of sleep-deprived young mice. This elevation subsequently improved synaptic plasticity, mitigated neuroinflammatory processes, and reversed cognitive impairment induced by sleep deprivation (Huang et al., 2025).

In recent years, the association between PC and depression has gained increasing attention. This study investigated how the microbiota in depressed mice modulates PC levels. Our data revealed decreased *Lactiplantibacillus* and increased *Anaerotignum* in depressed mice, accompanied by reduced colonic PC, and increased serum and hippocampal PC. *Lactiplantibacillus* abundance was negatively correlated with PC levels, whereas *Anaerotignum* showed a positive correlation. We propose that BP alleviates depression by: (1) modulating the balance between beneficial and pathogenic bacteria; and (2) normalising PC distribution across tissues. The observed PC redistribution was associated with antidepressant-like effects but mediation remains unproven. Future studies should directly manipulate candidate bacteria and PC availability to test this model. Consistent with our findings, Li et al. (Li, et al., 2021) reported that BP exerts antidepressant effects via microbiota–metabolite modulation, specifically by regulating amino acid, energy, and lipid metabolism to improve depressive behaviours. Lv et al. (Lv, et al., 2024) further demonstrated BP's efficacy in increasing *Lactobacillus* abundance and modulating tryptophan metabolism. Although we focused on the hippocampus due to its well-established role in stress responses and neuroplasticity, we acknowledge that other brain regions such as the prefrontal cortex (PFC) and nucleus accumbens (NAc) are equally central to the pathophysiology of depression. Future studies will extend our analysis to these brain regions to fully map the neuroanatomical landscape of BP's effects on phospholipid metabolism (Liu et al., 2021; Zhao, et al., 2024; Gao et al., 2025).

Correlation analysis identified associations among BP treatment, *Lactiplantibacillus* and *Anaerotignum* abundance, glycerophospholipid metabolites, and tissue PC levels. BP treatment was associated with lower hippocampal PC and higher colonic PC relative to the Mod group, whereas cecal PC content did not differ significantly. Notably, BP exerted a region-specific effect on intestinal PC metabolism: it significantly increased PC levels in colonic tissue but not in cecal contents. This differential response, which would have been missed if only one site had been analyzed, suggests that BP's mechanism is not to globally alter gut PC levels but rather to target the colonic epithelium to exert antidepressant effects. This observation underscores the value of spatial stratification in gut metabolomics and supports the notion that herbal medicines may act

through site-specific mechanisms along the gut–brain axis (Coleman et al., 2025; Chen et al., 2026; Spiga et al., 2026). This spatial pattern suggests a site-specific response but does not demonstrate colonic PC release, translocation to the hippocampus, or microbiota-dependent regulation. We hypothesise that BP extract: (1) inhibits colonic PC release into the circulation; (2) reduces PC translocation to the hippocampus; and (3) thereby alleviates depressive behaviours. This hypothesis requires direct tracing, microbial manipulation, and PC supplementation or depletion experiments. Accordingly, the observed PC redistribution should be interpreted as associated with, rather than mediating, the antidepressant-like effects of BP. Zhong et al. (Zhong et al., 2024) demonstrated that Sanghuang polyphenols restore gut barrier function, enrich *Alistipes onderdonkii*-derived 5-hydroxyindoleacetic acid, and activate aryl hydrocarbon receptor signalling to alleviate colitis. Yu et al. (Yu, et al., 2024) found that indole-3-acetic acid (IAA) crosses the blood–brain barrier via specific transporters. Similarly, ginsenoside Rg1 elevates *L. murinus*-derived IAA in blood and brain, enhancing hypothalamic oxytocin secretion and hippocampal neurogenesis. They proposed a dual mechanism: (i) physiological restoration of hippocampal neuronal PC regulation; and (ii) normalisation of colonic PC through gut barrier repair, immunomodulation, and gut–brain signalling. Specifically, BP-mediated modulation of gut microbiota structure may promote PC synthesis and homeostasis in colonic mucosal cells, thereby improving intestinal barrier function and ameliorating local inflammatory status (Van Der Veen et al., 2017). Concurrently, alterations in microbiota structure and the systemic effects of their metabolites may regulate hepatic lipid metabolism via the circulatory system. This regulation could reduce the delivery of the lipid-signalling molecule PC from serum to the central nervous system, indirectly downregulating the abnormal accumulation of PC in the hippocampal region under stress conditions (Li and Vance, 2008; Vance, 2008). Normalisation of hippocampal PC levels is proposed to contribute to the restoration of neuronal membrane fluidity, support the recovery of mitochondrial function and neurotransmitter balance, and ultimately alleviate depression-like behaviours (Paoletti et al., 2011; Fagone and Jackowski, 2013).

The current study had four main limitations concerning mechanistic depth and causal inference. First, a more detailed molecular understanding of BP's action is required; specifically, the mechanisms by which BP modulates key enzymatic activities in glycerophospholipid metabolism and related signalling networks remain to be fully uncovered. Future work will use targeted biochemical and genetic approaches, including western blotting, gene identification, and gene editing, to elucidate these upstream regulatory nodes. Second, and more critically, the causative links within the observed microbiota–metabolite interactions are unclear. Notably, FMT experiments were not conducted in the present setting, precluding definitive proof of the gut microbiota's role in depression aetiology and BP's efficacy. Future FMT-based studies will be essential to determine whether direct microbial manipulation drives metabolite changes, or vice versa, and to establish causality. Additionally, complementary *in vitro* and *in vivo* experiments will be performed: candidate bacterial strains, such as *Lactiplantibacillus* or *Anaerostignum*, will be cultured and transplanted into mice to investigate whether increasing their abundance modulates PC changes *in vivo*, further clarifying the causal chain from specific gut microbes to host metabolite alterations. Third, a key limitation of this study was the inability to establish a causal relationship between PC metabolic changes and antidepressant efficacy. Although we observed robust co-variation of PC in the intestine, serum, and hippocampus, these data alone cannot determine whether PC redistribution is a primary driver of the behavioural improvement. We cannot exclude the possibility that PC changes are correlated byproducts of other unelucidated mechanisms. To directly address this, we plan to conduct PC supplementation/depletion experiments in follow-up studies, such as supplementing dietary PC or using PC synthesis inhibitors (e.g., choline kinase inhibitors), to observe whether direct modulation of PC levels in CRS mice can mimic or block the effects of BP. Together, these planned experiments will systematically unravel the molecular mechanisms underlying the gut microbiota–metabolite–BP antidepressant axis. Finally, serum rather than plasma was used for untargeted metabolomics, and paired serum–plasma samples were not compared in this mouse cohort. Coagulation and platelet activation during serum preparation may alter lipid measurements, including phospholipid-related signals. Tight QC clustering supports instrument and pre-processing reproducibility only and cannot exclude this biological matrix bias. Future studies should

include paired serum–plasma comparisons under standardised processing conditions.

5 Conclusions

Our study identified an association between BP treatment and a candidate microbiota–gut–brain pattern involving *Lactiplantibacillus*, *Anaerotignum*, glycerophospholipid metabolism, and compartment-specific PC differences. Integration of 16S rRNA gene sequencing with serum metabolomics showed that microbial and metabolic changes co-occurred with antidepressant-like behavioural improvement. The observed PC redistribution was associated with antidepressant-like effects, but its direction, transport route, and mediating role were not established. These observations provide a framework for future studies using microbial manipulation, paired serum–plasma sampling, isotope tracing, and direct PC intervention. Our findings generated testable hypotheses. Causal relationships remain to be validated in further intervention experiments.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions

Yaru CUI drafted the manuscript. Minghui HU, Zifa LI, Lidan WU, and Hao ZHANG curated the data. Jialing XU, Yuxin TIAN, and Shimeng LV conducted the experimental investigations. Xinyu WANG reviewed and revised the manuscript. Sheng WEI conceived and supervised the study, acquired funding, and contributed to the original draft. All authors reviewed and approved the final manuscript.

Compliance with ethics guidelines

Yaru CUI, Minghui HU, Chao LIU, Jialing XU, Yuxin TIAN, Shimeng LV, Zifa LI, Lidan WU, Hao ZHANG, Xinyu WANG and Sheng WEI declare that they have no conflicts of interest. All procedures were approved by the Institutional Animal Care and Use Committee of Shandong University of Traditional Chinese Medicine (Approval No. SDUTCM20230403002).

Declaration on the use of generative AI tools

The authors declare that no generative AI tools were used in the preparation of this manuscript.

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Supplementary information

Methods; Figs. S1-S4