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Proteomics Shows the Role of *Paederia scandens* in Ameliorating Non-alcoholic Fatty Liver Disease in a Rat Model

(Proteomik Menunjukkan Peranan *Paederia scandens* dalam memulihkan Penyakit Hati Berlemak Bukan Alkohol pada Model Tikus)

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ABSTRACT

This study was conducted to evaluate the effect of *Paederia scandens* on high-fat dietinduced non-alcoholic fatty liver disease (NAFLD) in a rat model and further show the therapeutic mechanisms of *Paederia scandens*. Thirty rats weighing 180 ± 12 g (6 weeks old) were randomly divided into three groups: A control group (CG), a high-fat diet model group (HF), and a *Paederia scandens* intervention group (PS). After 45 days, the rats' serum lipid metabolism, liver injury parameters, and liver proteomics were detected. The results indicated that dietary *Paederia scandens* significantly reduced the levels of triglycerides, total cholesterol, glucose, and low-density lipoprotein cholesterol in the NAFLD rats compared with those in the HF group. Meanwhile, decreased levels of alanine aminotransferase and aspartate transaminase were observed in rats of the PS group. In addition, 382 differentially abundant proteins were identified between the HF and PS groups. Protein-protein interaction network analysis identified 14 keystone proteins that might play critical roles in ameliorating NAFLD. In particular, *Paederia scandens* treatment significantly upregulated the levels of Hadh, Hadhb, Acadl, Acox1, Acox3, Cyp3a2, and Cyp1a1, which are involved in fatty acid βoxidation, PPAR, and cytochrome P450 signaling pathways. Hence, the data demonstrated that *Paederia scandens* ameliorates hepatic lipid accumulation and impairment by enhancing fatty acid β-oxidation and activating PPAR and cytochrome P450 signaling pathways. These data provide new insights into the treatment of NAFLD and suggest the potential of *Paederia scandens* as an effective therapy.

Keywords: Non-alcoholic fatty liver disease; *Paederia scandens*; proteomics; rat; therapeutic mechanisms

ABSTRAK

Penyelidikan ini dijalankan untuk menilai kesan *Paederia scandens* terhadap penyakit hati berlemak bukan alkohol (NAFLD) akibat diet tinggi lemak pada model tikus dan seterusnya menunjukkan mekanisme terapeutik *Paederia scandens*. Tiga puluh ekor tikus dengan berat 180 ± 12 g (6 minggu) dibahagikan secara rawak kepada tiga kumpulan: Kumpulan kawalan (CG), kumpulan model diet tinggi lemak (HF) dan kumpulan intervensi *Paederia scandens* (PS). Selepas 45 hari, metabolisme lipid serum tikus, parameter kecederaan hati dan proteomik hati telah dikesan. Keputusan menunjukkan bahawa diet *Paederia scandens* secara signifikan mengurangkan tahap trigliserida, jumlah kolesterol, glukosa dan kolesterol lipoprotein berketumpatan rendah pada tikus NAFLD berbanding dengan kumpulan HF. Sementara itu, penurunan tahap alanine aminotransferase dan aspartate transaminase diperhatikan pada tikus kumpulan PS. Di samping itu, 382 protein yang banyak berbeza telah dikenal pasti antara kumpulan HF dan PS. Analisis rangkaian interaksi protein-protein mengenal pasti 14 protein kiston yang mungkin memainkan peranan penting dalam memperbaiki NAFLD. Khususnya, rawatan *Paederia scandens* dengan ketara mengimbangi tahap Hadh, Hadhb, Acadl, Acox1, Acox3, Cyp3a2 dan Cyp1a1 yang terlibat dalam pengoksidaan β asid lemak, PPAR dan laluan isyarat sitokrom P450. Oleh itu, data menunjukkan bahawa *Paederia scandens* memperbaiki pengumpulan dan kemerosotan lipid hepatik dengan meningkatkan pengoksidaan β asid lemak dan mengaktifkan laluan isyarat PPAR dan sitokrom P450. Data ini memberikan pandangan baharu tentang rawatan NAFLD dan mencadangkan potensi *Paederia scandens* sebagai terapi yang berkesan.

Kata kunci: Mekanisme terapeutik; penyakit hati berlemak bukan alkohol; *Paederia scandens*; proteomik; tikus

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is recognized as one of the most frequent chronic liver diseases, affecting a quarter of the population worldwide (Godoy-Matos, Silva Júnior & Valerio 2020). NAFLD is characterized by hepatic steatosis and is caused by increased liver fat accumulation in individuals without excessive alcohol consumption (Parker et al. 2012). Further inflammation of the liver might progress to fibrosis, cirrhosis, and hepatocellular carcinoma (Ahmed & Byrne 2009; Ahmed, Wong & Harrison 2015; Machado & Diehl 2016). Strong correlations between the development of NAFLD and metabolic syndromes, such as obesity, diabetes, insulin resistance, and dyslipidemia, have been established (Heindel et al. 2017). Currently, primary treatments for NAFLD are limited to lifestyle modifications, such as weight loss, dietary management, and physical activity, because there are no Food and Drug Administration (FDA)-approved drugs (Polyzos et al. 2020). However, previous studies have shown that after a 52-week long intensive intervention, including weight loss, dietary management, and physical activity, only 25% of patients achieved NAFLD resolution (Vilar-Gomez et al. 2015). Moreover, weight reductions of ≥ 10% are required to achieve efficacy; however, these are rarely maintained over a long-term (Romero-Gómez, Zelber-Sagi & Trenell 2017). Notably, the extent and intensity of lifestyle interventions varied widely in previous studies, and efficacy was inconsistent among individuals, making it difficult to convert lifestyle intervention trials into pivotal trials (Michel & Schattenberg 2020). Therefore, safe and effective drugs need to be evaluated and applied to treat NAFLD.

Traditional Chinese medicine has received widespread attention and is recognized as a complementary and alternative therapy (Li & Weng 2017). Traditional Chinese medicine has the characteristics of multi-component, low toxicity, and multitarget synergies for complex diseases in clinical application, which contrasts with singletarget chemical drugs with serious toxic side effects and poor therapeutic effects (Du et al. 2009; Zhang et al. 2016). Meanwhile, recent studies also indicated that Chinese herbs and their active ingredients could be used as potential therapeutic agents to treat NAFLD (Chen et al. 2021; Yan et al. 2020). *Paederia scandens* is a perennial herb of the Rubiaceae that is widely used in Vietnam, China, India, and Japan to treat toothache, hemorrhoids, and spleen infections (Trung et al. 2023). Recent research has found that *Paederia scandens* has hepatoprotective potential (Wu, Yang & Tang 2021; Xiao et al. 2019). Especially, iridoid glucosides isolated from *Paederia scandens* showed potent anti-inflammatory activities *in vitro* (Hou et al. 2014; Xu et al. 2023; Zhu et al. 2012). Meanwhile, the anti-inflammatory and hepatoprotective effects of *Paederia scandens* were observed in rats, comprising attenuated liver damage and reduced alanine aminotransferase (ALT) and aspartate transaminase (AST) levels (Peng et al. 2015). Furthermore, network pharmacology analyses were conducted to investigate the anti-inflammatory and hepatoprotective mechanisms of *Paederia scandens*, which indicated that it inhibited the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) and inducible nitrous oxide synthase (iNOS)/nuclear factor kappa B (NF-κB) signaling pathways to exerts anti-inflammatory effects (Chen et al. 2022; Tang et al. 2022). In addition, a study based on network pharmacology analysis found that *Paederia scandens* might regulate genes, such as *AKT1* (encoding AKT serine/threonine kinase 1), *IL10* (encoding interleukin 10), *CYP1A2* (encoding cytochrome P450 1A2), *CYP1A1* (encoding cytochrome P450 1A12), and *CYP3A4* (encoding cytochrome P450 3A4), to improve insulin resistance and oxidative stress in the pathogenesis of NAFLD (Zhu, Xiao & Li 2019). These studies demonstrated the antiinflammatory and hepatoprotective properties of *Paederia scandens*. Notably, inflammatory cytokines are involved in the development and progression of NAFLD, and have been used as biomarkers to assess the severity and predict the prognosis of NAFLD (Duan et al. 2022). Therefore, *Paederia scandens* might play a role in the treatment of NAFLD.

However, there is a lack of validation of *Paederia scandens* in an NAFLD model and the targets of its hepatoprotective effects require further exploration in animal models. Currently, proteomics is recognized as an important approach to improve the study of the complex pathogenesis of NAFLD, especially in pathophysiological assays and the identification of new markers for disease diagnosis (Aslam et al. 2016; Nuño-Lámbarri et al. 2016). Therefore, the present study used an NAFLD rat model, aiming to show the therapeutic mechanisms of *Paederia scandens* using proteomics. Our findings will provide new insights into the treatment of NAFLD and encourage further translational and clinical trials of *Paederia scandens*.

MATERIALS AND METHODS

ANIMAL MANAGEMENT AND EXPERIMENTAL TREATMENTS

The 30 Sprague-Dawley male rats weighing 180 ± 12 g (6 weeks old) used in this experiment were supplied by Cyagen Biosciences Co., Ltd (Jiangsu Province, China). The experimental animals were randomly divided into three groups: The control group (CG), the high-fat diet model group (HF), and the *Paederia scandens* intervention group (PS). The rats in the CG group were fed with a normal diet

(Table 1), whereas the rats in the other groups were supplied with a high-fat diet (the normal diet supplemented with 10% lard oil) and injected intraperitoneally with 100 mg/kg thioacetamide to induce NAFLD. The mechanism by which thioacetamide injection induces NAFLD involves hepatocyte uptake and conversion of thioacetamide to reactive metabolites, which cause oxidative necroinflammation, and excessive activation and proliferation of collagen-producing hematopoietic stem cells (Hansen et al. 2017). In addition, the rats in the PS group were supplemented with the 5% *Paederia scandens* at a dosage of 1 mL/g to investigate its therapeutic effect. The doses of *Paederia scandens* were determined according to references and the results of previous experiments (Peng et al. 2015). Diets and water were supplied *ad libitum* for all rats. All rats were raised individually in a ventilative squirrel cage (23 ± 2 °C, humidity 50 ± 10 %, 12 h light/dark cycle). All rats were adapted to the experimental environment for 7 days before the formal trial, which lasted for 45 days.

Before sampling at the end of the experiment, all the rats were fasted for 12 h and then sacrificed by intraperitoneal injection with 50 mg/kg sodium pentobarbital; sterile conditions were maintained during the operation. Then, a laparotomy was performed to expose the celiac artery and liver, and blood was collected from the celiac artery into tubes without anticoagulants. Furthermore, the livers of the rats were excised and stored at -80 °C for the subsequent experiments (Yu et al. 2018). The animal experiments were performed in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The experimental protocol and procedures were authorized by the Animal Care and Use Committee of Yibin Vocational and Technical College (permit no. AEC-YVTC-20221008).

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) DETECTION

To determine the contents of the main components of *Paederia scandens*, linalool, borneol, methylsalicylate, eugenol, d-a-terpineol, isoeugenol, l-a-terpineol, and camphor contents were analyzed using an Agilent 1260 HPLC system equipped with an Agilent Zorbax Eclipse XDB-C8 column (4.6 mm × 250 mm, 5 μm; Agilent Technologies, Santa Clara, CA, USA). The detection conditions comprised a 10 μL injection volume with a flow rate of 1.0 mL/min. In addition, the detection wavelength was 230 nm and the column temperature was 30 °C.

DETERMINATION OF SERUM PARAMETERS

The collected blood was centrifuged at 3000 × g for 15 min at 4 °C to obtain serum, which was used to determine lipid metabolism and liver injury parameters. In detail, triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDL-C), glucose (GLU), ALT, AST, albumin (ALB), and globulin (GLOB) were measured using a KHB400 automatic biochemical analyzer (Kehua Bioengineering, Co. Ltd., Shanghai, China) based on the manufacturer's instructions and according to a previous study (Dai et al. 2022).

ISOBARIC TAGS FOR RELATIVE AND ABSOLUTE QUANTITATION (ITRAQ) ANALYSIS

Protein were extracted from 500 mg of liver tissue for iTRAQ analysis according to a previously described method (Deng et al. 2018). The liver tissue was put into a 1.5 mL centrifuge tube with 5 mm magnetic beads and lysis buffer. Then, phenylmethanesulfonyl fluoride was

TABLE 1. Composition and nutrient levels of the diets

Ingredients (%)		Nutritional level (%)	
Corn	60.74	ME (Mcal/kg)	3.02
Soybean meal	34.50	CP	20.12
Fried meal	1.20	Ca	0.88
Salt	0.30	P	0.62
Lys	0.20		
Met	0.26		
$CaHPO_4$	1.30		
Limestone	1.20		
Additives	0.30		

Per kg, the diet contains Fe 80 mg, Zn 40 mg, Cu 8 mg, Mn 60 mg, I 0.35 mg, Se 0.15 mg, 0.20% of choline, vitamin (V)A 1500IU, VD3 200IU, VE 10IU, VK3 0.05 mg, VB1 1.80 mg, VB2 3.60 mg, VB12 0.01 mg, VB7 0.15 mg, VB9 0.55 mg, VB5 35 mg, VB3 10 mg, VB6 3.50 mg. The additive contains 0.50% of microelements

added at a final concentration of 1 mM and ethylenediamine tetraacetic acid (EDTA) was added at 2 mM. After adding DLdithiothreitol (DTT) at a final concentration of 10 mM, a tissue grinder was used break the tissue with oscillations for 2 min at 50 Hz. The solution was centrifuged at 25,000 × g for 20 min at 4 °C to obtain the supernatant. A final concentration of 10 mM DTT was added to the supernatant and incubated for 1 h in a water bath at 56 °C. After cooling to room temperature, iodoacetamide (IAM) was added at a final concentration of 55 mM and the solution was left for 45 min in the dark. Thereafter, four volumes of cool acetone were added and the mixture was placed at –20 °C for 2 h. This operation was repeated two or three times until the supernatant was colorless. Finally, the solution was centrifuged at 25,000 × g for 20 min at 4 °C and the supernatant was discarded. Then, 5 mm magnetic beads and the appropriate amount of lysis buffer were added to the precipitate and oscillated using a tissue grinder for 2 min at 50 Hz. Finally, the solution was centrifuged at 25,000 × g for 20 min at 4 °C to obtain the supernatant and a bicinchoninic acid (BCA) kit was used for protein quantification.

For each protein sample, 100 μg of protein was taken and 2.5 μg of Trypsin was added at a mass ratio of 40:1 (protein:enzyme) for digestion at 37 °C for 4 h. The peptide samples were then labeled using iTRAQ-8plex labeling reagents, which were incubated at room temperature for 2 h. The peptides were fractionated on a Shimadzu LC-20AB HPLC system (Shimadzu Corporation, Kyoto, Japan) using a 5 μm 4.6 × 250 mm Gemini C18 column. Peptides were eluted at a flow rate of 1 mL/min and a linear gradient of 5% solvent B for 10 min. The eluted peptide samples were redissolved in solvent A (2% acetonitrile (ACN), 0.1% formic acid (FA)), centrifuged at 20,000 × g for 10 min, and then the supernatant retained as the sample. After separation performed using the Shimazu LC-20AD HPLC system (Shimadzu Corporation), the peptides were ionized using a nano electrospray ionization (ESI) and processed using a Q-Exactive mass spectrometer (Thermo Fisher Scientific, San Jose, CA) for datadependent acquisition mode detection.

BIOINFORMATIC ANALYSIS

IQuant software was used to quantify the iTRAQ data (Wen et al. 2014) and data integration was carried out using the Mascot Percolator algorithm (Brosch et al. 2009). First, spectra and peptide lists were obtained using false-positive rate (FDR) filtering (FDR ≤ 0.01). Then, the peptides were assembled to generate the proteome. To control the FDR of proteins, the protein level was filtered again at 1% FDR using the picked protein FDR (Savitski et al. 2015). Proteins with a fold change > 1.2 or < 0.83 and a q-value < 0.05 were considered as differentially abundant proteins. The protein functional enrichment analysis was performed using the Cluster of Orthologous Groups of proteins (COG) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) databases. In addition, the protein-protein interaction (PPI) network of the differentially abundant proteins (combined score > 0.90) was constructed using the STRING database (Szklarczyk et al. 2016). The proteins with a higher betweenness centrality and degree were regarded as the keystone proteins in the PPI network.

STATISTICAL ANALYSIS

To evaluate the statistical significance, SAS Version 9.2 (SAS Institute Inc., Cary, NC, USA) was used for one-way analysis of variance (ANOVA), Duncan's multiple range test, and normality tests. Data with $P < 0.05$ was considered statistically significant.

RESULTS

CHEMICAL COMPOSITION OF *Paederia scandens*

The major components of *Paederia scandens* were identified using HPLC. Table 2 shows that the component with the highest content in *Paederia scandens* was linalool (261.142 mg/mL), followed by borneol (118.784 mg/mL), and methylsalicylate (78.902 mg/mL). Moreover, the contents of eugenol, d-a-terpineol, isoeugenol, l-a-terpineol, and camphor in *Paederia scandens* were relatively low (10-25 mg/mL).

EFFECTS OF *Paederia scandens* ON LIPID METABOLISM AND LIVER INJURY PARAMETERS

Lipid metabolism and liver injury parameters were measured to assess successful establishment of the rat model of NAFLD and the effect of *Paederia scandens* on the induced NAFLD (Table 3). Compared with the CG group, the contents of TG, TC, LDL-C, and GLU in the serum of rats in the HF group were significantly increased ($P < 0.05$). In addition, higher levels of ALT and AST were observed in the HF rats compared with those in the CG rats ($P < 0.05$). These results indicated that the rat model of NAFLD was successfully established. In addition, compared with those in the HF group, we found that dietary *Paederia scandens* significantly reduced levels of TG, TC, LDL-CH, and GLU in the serum of rats fed with the high-fat diet ($P < 0.05$). Furthermore, decreased levels of ALT and AST were observed in the serum of rats in the PS group.

TABLE 2. Contents of the major components in Paederia scandens

Ingredients	Content (mg/mL)
Linalool	261.142
Borneol	118.784
Methylsalicylate	78.902
Eugenol	24.634
D-a-terpineol	11.233
Isoeugenol	21.346
L-a-terpineol	10.126
Camphor	15.234

TABLE 3. Effects of Paederia scandens on lipid and glucose metabolism and liver injury parameters

Items	CG	HF	PS
TG, mmol/L	0.35 ± 0.01^{b}	0.52 ± 0.15^{a}	0.39 ± 0.16^{b}
TC, mmol/L	1.38 ± 0.47^{b}	2.22 ± 0.36^{a}	1.46 ± 0.17^{b}
HDL-C, mmol/L	0.76 ± 0.06	0.66 ± 0.26	0.76 ± 0.08
LDL-C, mmol/L	0.33 ± 0.01^{c}	0.87 ± 0.15^{a}	0.61 ± 0.12^{b}
GLU, mmol/L	5.38 ± 0.39^{b}	6.14 ± 0.78^{a}	5.64 ± 0.44^{b}
ALT, U/L	48.78 ± 13.24^{b}	78.97 ± 24.64^{a}	59.50 ± 14.88^{ab}
AST, U/L	153.74 ± 22.16^{b}	222.53 ± 47.30^{a}	174.40 ± 40.03^{b}
ALB, g/L	25.21±2.19	28.3±1.87	30.37±0.97
GLOB, g/L	44.8±3.53	41.27±2.06	42.13±2.51

[a,b,c] Means within a row with no common superscript differ significantly (n = 10; $P < 0.05$). CG, control group; HF, high-fat diet; PS, high-fat diet with *Paederia scandens* intervention. TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; GLU, glucose; ALT, alanine aminotransferase; AST, aspartate transaminase; ALB, albumin; GLOB, globulin

CHARACTERIZATION OF DIFFERENTIALLY ABUNDANT PROTEINS AND THEIR FUNCTIONS IN THE LIVER

A total of 28793 peptides and 5897 proteins were identified in the iTRAQ assay. By comparison with the COG database, the functions of these identified proteins are mainly annotated to cellular programming, which is involved in protein translation and posttranslational modification, signal transduction mechanisms, transcription, replication, recombination, and repair (Figure 1). In addition, a total of 382 differentially abundant proteins were identified between the HF and PS groups (fold change > 1.2 and q-value < 0.05). Compared with the HF group, we found that 185 proteins were significantly upregulated, while 197 proteins were significantly downregulated in the PS group (Figure 2). Furthermore, we found that the differentially abundant proteins were significantly enriched in 20 pathways according to analysis in the KEGG database (Figure 3). Pentose and glucuronate interconversions, steroid hormone biosynthesis, fatty acid degradation, glutathione metabolism, and peroxisome proliferator-activated receptors (PPAR) signaling pathways are the major pathways involved in the regulation of host glycolipid metabolism. In addition, some pathways regulating the inflammatory response were significantly enriched, including ascorbate and alternate metabolism,

retinol metabolism, IgA production, and the PPAR signaling pathway. Notably, chemical carcinogenesis, primary immunodeficiency, allograft rejection, autoimmune thyroid disease, asthma, African trypanosomiasis, and viral myocarditis, which are involved in metabolic diseases, were also enriched.

PPI NETWORK ANALYSIS OF THE DIFFERENTIALLY ABUNDANT PROTEINS

Interactions of the differentially abundant proteins were analyzed using the STRING database, as shown in Figure 4. A total of 14 keystone proteins were identified that might play critical roles in ameliorating NAFLD (Table 4). In particular, Hadh, Hadhb, Acox1, Acadl, Acox3, Echdc1, Pccb, and Hsd17b6 are responsible for regulating lipid transport and metabolism. In addition, Agxt2, Abat, Cyp3a2, Cyp1a1, Gstt1, and Aox1 are involved in modulating amino acid transport and metabolism, secondary metabolites biosynthesis, transport and catabolism, posttranslational modification, protein turnover, chaperones, and nucleotide transport and metabolism, respectively (Table 4). These results showed the potential mechanisms by which *Paederia scandens* improves NAFLD via the regulation of host physiological metabolism.

DISCUSSION

In general, disorders of lipid metabolism and abnormal accumulation of lipids in the liver caused by excessive intake of high-fat diets are the major triggers of NAFLD (Lian et al. 2020). In the present study, the data suggested that the protective effects of *Paederia scandens* on NAFLD are mediated by its effective modulation of hepatic lipid metabolism to reduce lipid accumulation. The dynamic balance of hepatic fat is disrupted and the ability of the liver to oxidize fatty acids is reduced in insulin resistance, ultimately causing the abnormal accumulation of hepatic lipids in the liver (Gu et al. 2005). Increased TG and TC levels are regarded as characteristic of abnormal accumulation of hepatic lipids in a host with NAFLD (Liu, Bengmark & Qu 2010). Indeed, the result of this study found that dietary *Paederia scandens* significantly decreased the contents of TG and TC in the serum of rats with NAFLD. Consistently, a previous study reported that *Paederia scandens* decreased the contents of TG in a chicken NAFLD model (Wu, Yang & Tang 2021). Linalool, one of the primary components of *Paederia scandens*, can downregulate the expression of 3-hydroxy-3-methylglutaryl CoA reductase through sterol regulatory element binding protein 2 and ubiquitin-dependent mechanisms, thereby, reducing the production of lipids and cholesterol (Cheng, Sheen & Chang 2018; Cho et al. 2011). This could

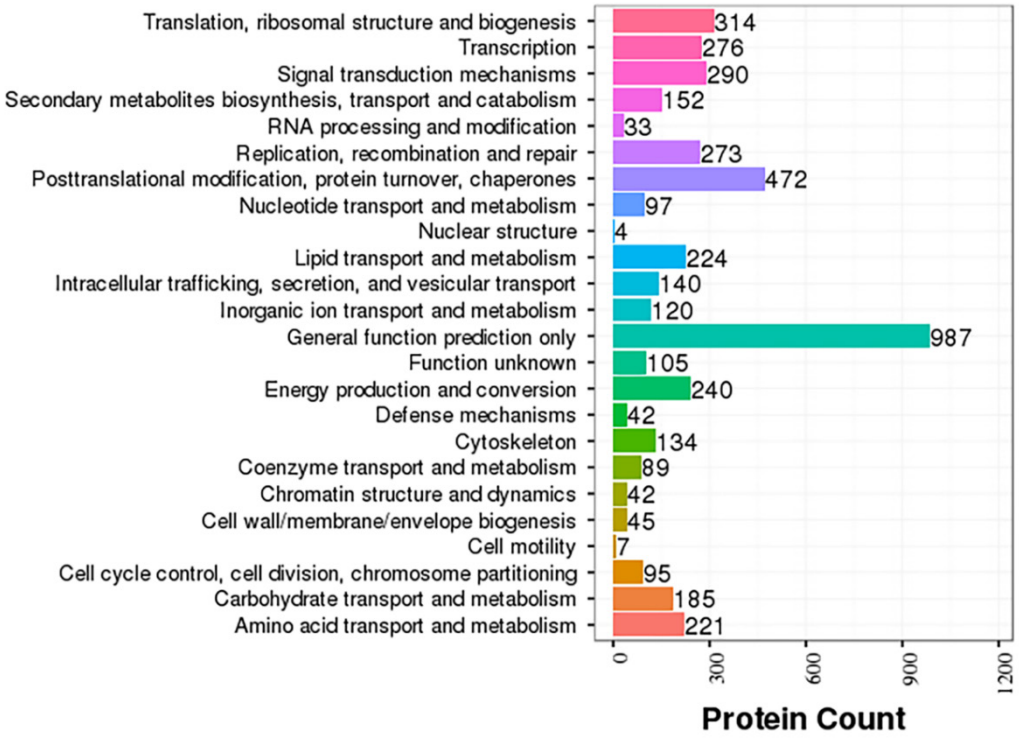

FIGURE 1. COG functional classification of proteins identified using proteomics

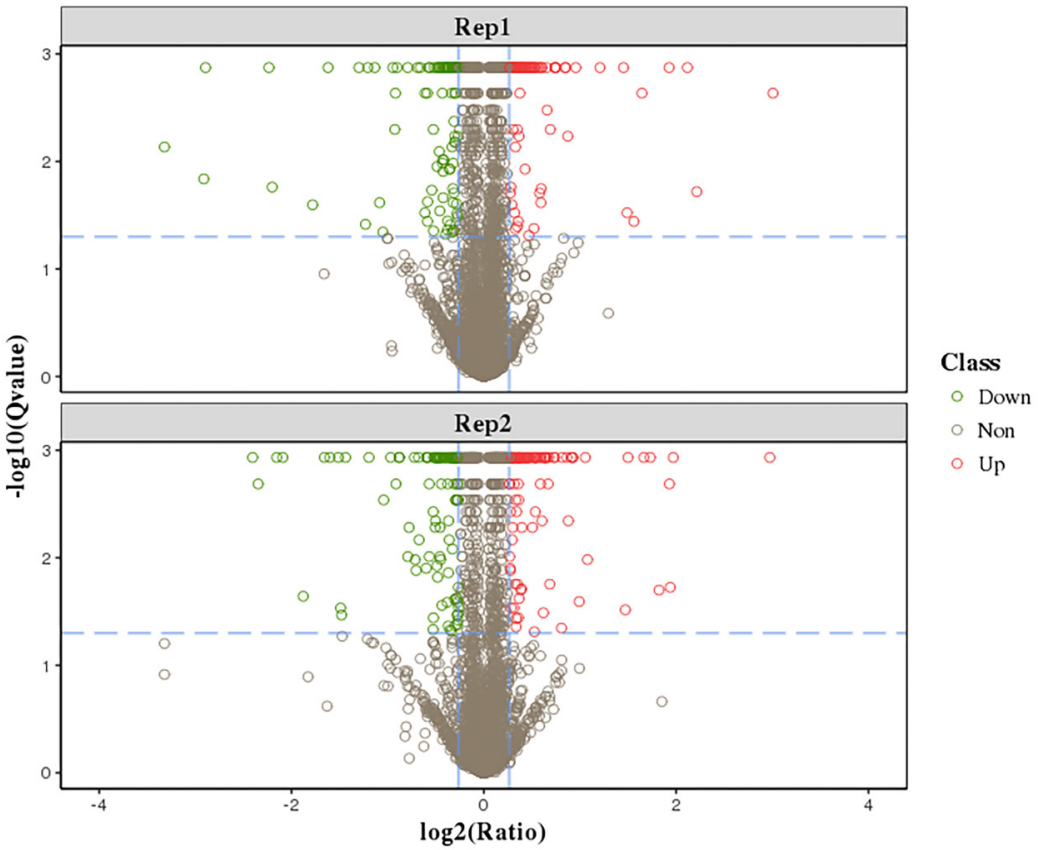

FIGURE 2. The volcano map of the proteomics-identified differentially abundant proteins

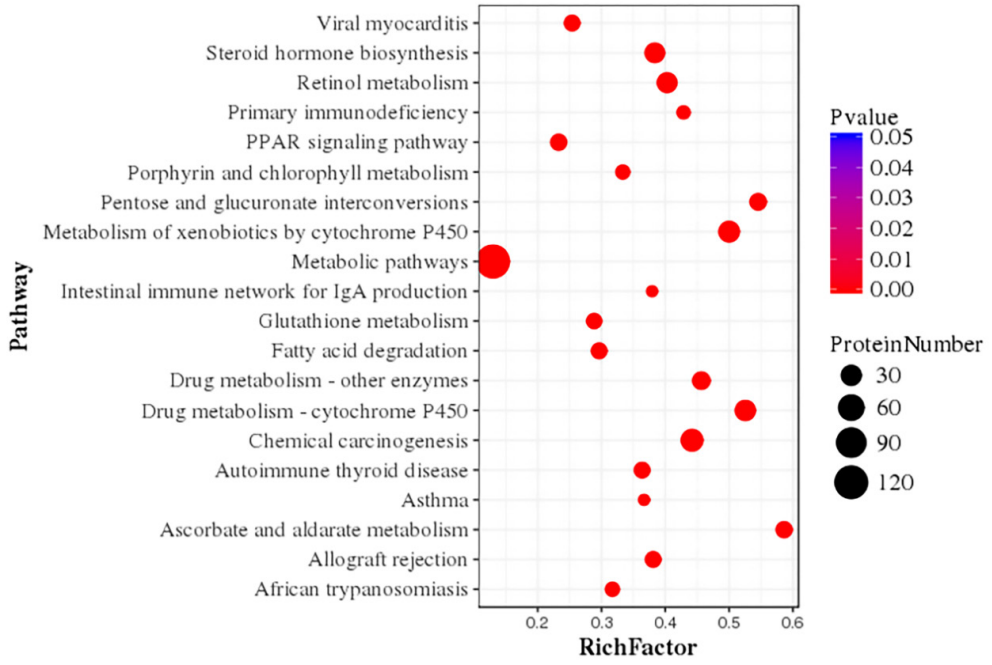

FIGURE 3. Pathway enrichment analysis of the differentially abundant proteins identified using proteomics

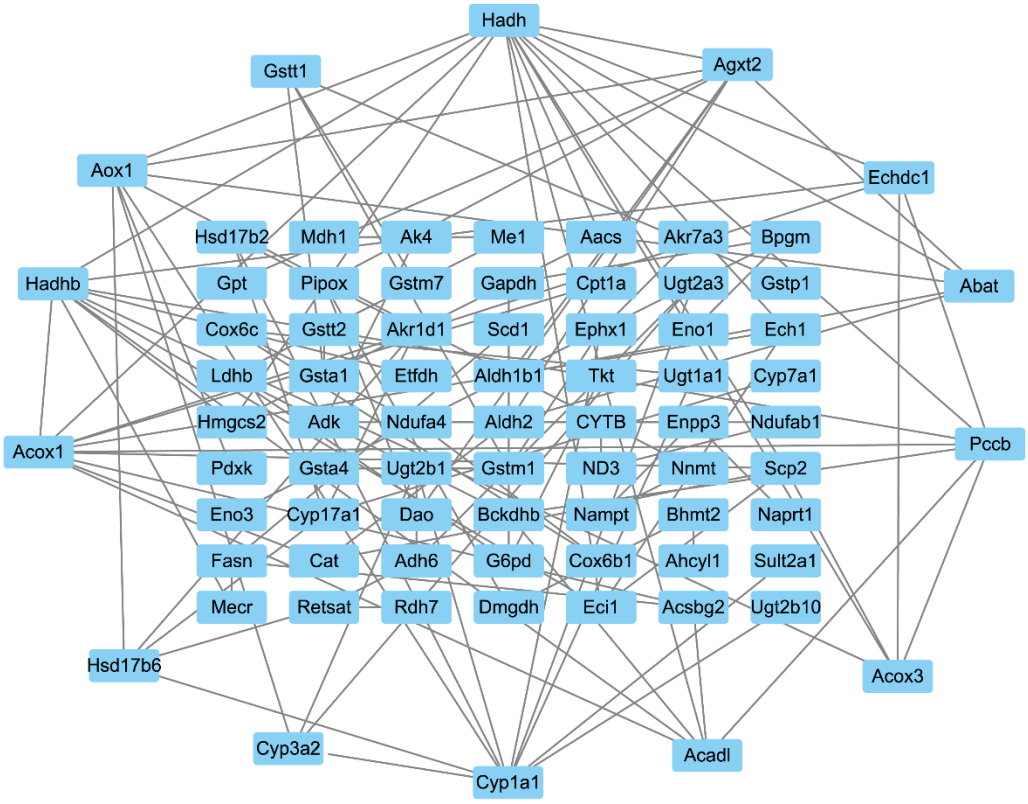

FIGURE 4. The protein-protein interaction network analysis of differentially abundant proteins identified using proteomics

partly explain the effect of *Paederia scandens* on the TG and TC levels in hepatic lipid metabolism. In the current study, dietary *Paederia scandens* also modulated the Cytochrome P450 signaling pathways, in particular by significantly upregulating Cyp3a2 and Cyp1a1 protein levels, to maintain homeostasis of the lipid metabolism in NAFLD rats. Important roles of Cytochrome P450s in the maintenance of lipid homeostasis haves been reported, in which they initiate all primary pathways of cholesterol degradation, including cholesterol, vitamin D, oxysterol, and bile acid metabolism (Hafner, Rezen & Rozman 2011). Dietary regulation strategies can activate nuclear receptors, such as the liver X receptor, farnesoid X receptor, and Vitamin D receptor, to eliminate cholesterol by conversion to bile acids (Chen, Thomsen & Vitetta 2019; Hafner, Rezen & Rozman 2011; Ma & Patti 2014). Therefore, activation of Cytochrome P450 pathways might be a potential mechanism underlying the effect of *Paederia scandens* on the TC content of NAFLD rats.

In addition, prolonged disruption of lipid metabolism can induce excessive production of reactive oxygen species in the liver, which further induces lipid peroxidation and depletion of endogenous antioxidant enzymes, resulting in serious damage to liver cells (Krifka et al. 2013; Wu et al. 2020; Zhou et al. 2019). In the diagnosis of NAFLD, AST, and ALT are common indicators used to evaluate hepatic injury and function (Gholam et al. 2007). In the current study, the elevated AST and ALT levels indicated that the liver had been damaged in the NAFLD rats, which further confirmed that a NAFLD model with liver injury was established in the rats. Surprisingly, we found that dietary *Paederia scandens* significantly reduced AST and ALT levels in NAFLD rats, suggesting an ameliorative effect of *Paederia scandens* on liver damage, which was consistent with the results of previous studies (Peng et al. 2015; Tang et al. 2022). Numerous studies have reported that the hepatoprotective activity of linalool alleviates liver injury by regulating the antioxidant defense system

TABLE 4. Profiles of 14 differentially abundant keystone proteins (up, upregulated; down, downregulated)

Protein ID	Description	COG description	COG categories	KEGG	Change
Hadh	Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial	3-hydroxyacyl-CoA dehydrogenase	Lipid transport and metabolism	K00022	up
Hadhb	Trifunctional enzyme subunit beta, mitochondrial	Acetyl-CoA acetyltransferase		K07509	up
Acox1	Acyl-coenzyme A oxidase	Acyl-CoA dehydrogenases		K00232	up
Acadl	Long-chain specific acyl-CoA dehydrogenase, mitochondrial			K00255	up
Acox3	Acyl-coenzyme A oxidase			K00232	up
Echdc1	Ethylmalonyl-CoA decarboxylase	Enoyl-CoA hydratase/carnithine racemase		K18426	up
Pccb	Propionyl coenzyme A carboxylase, beta polypeptide	Acetyl-CoA carboxylase, carboxyltransferase component (subunits alpha and beta)		K01966	down
Hsd17b6	17-beta-hydroxysteroid dehydrogenase type 6	Dehydrogenases with different specificities (related to short-chain alcohol dehydrogenases)		K13369	down
Agxt2	Alanine--glyoxylate aminotransferase 2, mitochondrial	4-aminobutyrate aminotransferase and related aminotransferases	Amino acid transport and metabolism	K00827	up
Abat	4-aminobutyrate aminotransferase, mitochondrial			K13524	down
Cyp3a2	Cytochrome P450	Cytochrome P450	Secondary metabolites biosynthesis, transport and catabolism	K07424	up
Cyp1a1	Cytochrome P450 1A1			K07408	up
Gstt1	Glutathione S-transferase theta 1	Glutathione S-transferase	Posttranslational modification, protein turnover, chaperones	K00799	up
Aox1	Aldehyde oxidase 1	Xanthine dehydrogenase, molybdopterin-binding subunit B	Nucleotide transport and metabolism	K00157	down

(Altınok-Yipel et al. 2019; Mazani et al. 2022; Ola & Sofolahan 2021). Moreover, accumulating evidence indicates that borneol, one of the main ingredients of *Paederia scandens*, is responsible for mitigating inflammation in the liver (Liu et al. 2023; Madhuri & Naik 2017). In summary, *Paederia scandens* plays an essential role in the regulation of lipid metabolism and the improvement of liver inflammation in NAFLD rats.

Although the modulatory effects of *Paederia scandens* on liver lipid metabolism and damage have been observed in a previous study (Zhu, Xiao & Li 2019), the specific mechanism is not clear. The current study showed the ability of *Paederia scandens* to regulate lipid metabolism by stimulating fatty acid β-oxidation to enhance fatty acid degradation in rats with NAFLD. Our data showed that proteins involved in fatty acid β-oxidation, including Hadh (3-hydroxyacyl-CoA dehydrogenase), Hadhb (acetyl-CoA acyltransferase), Acadl (long-chain specific acyl-CoA dehydrogenase), Acox1 (Acyl-coenzyme A oxidase), and Acox3 (Acyl-coenzyme A oxidase), were upregulated in rats fed with *Paederia scandens* compared with those in the model group, which indicates that fatty acid β-oxidation contributes to the effect of *Paederia scandens* on the decreased level of TG in NAFLD. In fact, the enhancement of fatty acid β-oxidation to promote the degradation of fatty acids and inhibition of fatty acid biosynthesis are common treatments for NAFLD (Fuchs, Traussnigg & Trauner 2016; Wang et al. 2021). In addition, our data showed that *Paederia scandens* also activated the PPAR signaling pathway, including Acadl and Acox1. PPAR is considered to be an energy receptor and can inhibit fatty acid biosynthesis by regulating the liver X receptor and sterol regulatory element binding protein (Yoshikawa et al. 2003). Studies have shown that PPAR is highly expressed in the liver of obese patients, suggesting that the accumulation of lipids is closely associated with increased hepatic fatty acid synthesis (Pyper et al. 2010). Besides, PPARα also promotes β-oxidation of fatty acids in mitochondria and reduces the deposition of lipids in the liver (Minnich et al. 2001; Pyper et al. 2010). Notably, PPAR ligands play a critical role in the anti-inflammatory response, which can inhibit the recruitment of leukocytes and downregulate cytokine and chemokine secretion (Costa et al. 2011; Straus & Glass 2007). This suggests that the alleviating effect of *Paederia scandens* on liver injury might be mediated through the PPAR signaling pathway. Notably, our study is the first to show that the protection provided by *Paederia scandens* is mediated by activating PPAR and Cytochrome P450 signaling pathways, which subsequently ameliorate abnormal lipid metabolism and impairment of the liver of NAFLD rats. Overall, enhanced fatty acid β-oxidation and activation of PPAR and Cytochrome P450 signaling pathways appear to largely responsible for the reduced accumulation of lipids in NAFLD rats fed with *Paederia scandens*. However, our study still has potential limitations, such as lacking validation of the relevant signaling pathways using molecular biology methods. Further research is required to evaluate the safety of *Paederia scandens*, which is essential for its application in the treatment of NAFLD.

CONCLUSIONS

This study explored the role of *Paederia scandens* in ameliorating NAFLD using proteomics in a rat model. Our findings indicated *Paederia scandens* regulates lipid metabolism to reduce the accumulation of liver lipids in NAFLD rats. Furthermore, proteomic analyses showed that *Paederia scandens* upregulated Hadh, Hadhb, Acadl, Acox1, and Acox3 protein levels to enhance fatty acid β-oxidation and degradation by activating PPAR and Cytochrome P450 signaling pathways, which subsequently ameliorated the abnormal lipid metabolism and hepatic impairment in NAFLD rats.

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