Cyclocarya paliurus Leaves Tea Improves Dyslipidemia in Diabetic Mice: A Lipidomics-Based Network Pharmacology Study

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Hyperlipidemia and hepatic steatosis afflict over 75% of patients with type 2 diabetes, causing diabetic dyslipidemia. Cyclocarya paliurus (CP) leaf is a herbal tea which has long been consumed by the Chinese population, particularly people suffering from obesity and diabetes. CP appears to exhibit a hypolipidemic effect in lipid loaded mice (Kurihara et al., 2003), although the detailed mechanisms and active ingredients for this hypolipidemic effect have not yet been answered. In this study, we investigated the beneficial effects of CP and predicted the mechanisms by utilizing lipidomics, serum-pharmacochemistry and network pharmacology approaches. Our results revealed that serum and hepatic levels of total triglyceride (TG), total cholesterol (T-CHO), low-density lipoproteins (LDL) and high-density lipoproteins (HDL), as well as 30 lipids including cholesterol ester (CE), diglyceride (DG), phosphatidylethanolamine (PE), phosphatidylcholine (PC), and sphingomyelin (SM) in CP-treated mice were improved in comparison with untreated diabetic mice. In parallel, 14 phytochemical compounds of CP were determined in mice serum after CP administration. Mechanistically, the network pharmacology analysis revealed the predicted targets of CP’s active ingredients ALOX12, APP, BCL2, CYP2C9, PTPN1 and linked lipidome targets PLD2, PLA2G(s), and PI3K(s) families could be responsible for the CP effects on diabetic dyslipidemia. In conclusion, this study revealed the beneficial effects of CP on diabetic dyslipidemia are achieved by reducing accumulation of hepatic lipid droplets and regulating circulatory lipids in diabetic mice, possibly through PI3K signaling and MAPK signaling pathways.

Keywords: Cyclocarya paliurus, Diabetic dyslipidemia, hyperlipidemia, lipidomic, network pharmacology

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, urea nitrogen; CE, cholesterol ester; CP, Cyclocarya paliurus; CREA, creatinine; DG, diglyceride; HDL, high-density lipoproteins; LDL, low-density lipoproteins; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PPI, protein–protein interaction; SM, sphingomyelin; STZ, streptozocin; T-CHO, total cholesterol; TG, total triglyceride.
INTRODUCTION

Hyperlipidemia and hepatic steatosis are frequently found in the metabolic syndrome and type 2 diabetes (Jung and Choi, 2014; Perry et al., 2014). Hyperlipidemia is characterized as high T-CHO, TG, LDL, and low HDL levels (Richhariya et al., 2015), whilst hepatic steatosis is represented by high TG, T-CHO, AST, and ALT levels (Stern and Castera, 2017). Emerging evidence suggest that dyslipidemia is a significant risk factor for the development of type 2 diabetes (Andriankaja and Joshipura, 2014; Association, 2015), and pharmacological lipid-lowering therapy is effective to alleviate the complications of type 2 diabetes including hyperlipidemia, hepatic steatosis, coronary heart disease, etc. (Zafrir and Jain, 2014; Sattar et al., 2016; Spence et al., 2016). Cyclocarya paliurus (Batal.) Iljinskaja (Juglandaceae) is a native medicinal plant grown in the southwest of China. The leaves of C. paliurus (CP) have been used as a herbal tea in China for its special flavor and the benefits to the obese and diabetic Chinese populations. The bioactive components isolated from CP are flavonoids, triterpenoids, organic acids, and polysaccharides, these components contribute to its versatile biological properties including antihyperglycemia, antihyperlipidemia, and antihypertension (Jiang et al., 2015; Yoshitomi et al., 2017). Previous studies have investigated the anti-diabetic function and mechanism of CP leaves on STZ and high-fat diet-induced type 2 diabetic mice, and its potent hypoglycemic effect has been verified on diabetic mice (Wang Q. et al., 2013; Ma et al., 2015). Moreover, CP has also been reported with hypolipidemic effects in vivo (Yao et al., 2015; Lin et al., 2016; Yang et al., 2016). However, the mechanism of bioactive constituents of CP on diabetic dyslipidemia remain unknown. Therefore, our objective is to study the effects of CP on lipid disorders in diabetes and elucidate its mechanism-of-actions.

It is acknowledged that the majority of herbal medicines, including TCMs are orally delivered drugs of polypharmacy. Their active components are firstly absorbed into the bloodstream and then selectively and simultaneously interact with multiple targets at the root causes of the disease (Zhao et al., 2015). Serum pharmacochemistry is a rapid and reliable method using the UPLC-MS technique to track the components absorbed into the bloodstream and has been widely used to reveal the efficacy of TCMs (Yan et al., 2017). Moreover, metabolomics is an approach to analyze the metabolites, the intermediate products of metabolic reactions of living systems. This technology contains many subclasses based on the chemical characteristics of metabolites, namely lipidomics, amino metabolomics, and sugar metabolomics, etc. It has been widely used in the evaluation of the therapeutic effects and elucidation of the therapeutic mechanisms of herbal products (Wang X. et al., 2017). Metabolomics profiling can reveal whole metabolic profile changes of living systems in response to endogenous or exogenous stimuli such as drug treatment (Beger et al., 2016). Because any effects of herbal products are mediated by their constituents in a complex biological system, metabolomics can help us to analyze their action network comprehensively. Network pharmacology is a bioinformatics strategy to understand drug action and mechanisms by mapping drug-target-disease networks from the biological level (Li et al., 2014). To date, accumulating evidence suggest that network pharmacology approach is a powerful tool to study the molecular mechanisms of the complex components found in medicinal herbs.

Considering CP as a herbal tea with multiple components and diabetic dyslipidemia as a cluster of lipid abnormalities, we focused on the investigation of the beneficial effect of CP and its mechanisms against diabetic dyslipidemia using lipidomics, serum pharmacochemistry, and network pharmacology approaches.

MATERIALS AND METHODS

Chemicals and Materials
Regents and Standards
UPLC grade organic solvent was purchased from Merck (Darmstadt, Germany). Deionized water was obtained from a
milled, ammonium formate, and phosphoric acid were obtained from Sigma (St. Louis, MO, United States). Lipids standards including a lipids mixture of TG, CE, DG, PE, PC, and SM, etc., were purchased from Avanti Polar Lipid (Alabaster, AL, United States). Strptomycin and glibenclamide was purchased from Sigma (St. Louis, MO, United States).

**Plant Material Preparation**

The leaves of *Cyclocarya paliurus* (Batal.) Iljinskaja were collected and authenticated by Prof. Hu-Biao Chen from School of Chinese Medicine, Hong Kong Baptist University, and voucher specimens (No. CP20151201) was stored in our Research Laboratory. For the preparation of the extract of *C. paliurus* leaves (CP extract), the dried leaves of *C. paliurus* (5 kg) were soaked in boiled water (1:10 m/v) for 2 h twice. The solution was concentrated and dried under vacuum to obtain crude extract. The crude extract was then extracted by 70% EtOH for 2 h (1:10 m/v) in an ultrasound bath. The refined solution was concentrated and dried under vacuum again and the refined extract was stored in 4°C fridge until use. CP solution was prepared with 0.5% sodium carboxymethyl cellulose (CMC-Na) solution for animal oral administration and MeOH for phytochemical analysis.

**Animal Studies**

**Animal Handling and Diets**

Eight-week-old C57/BL6J mice were purchased from Laboratory Animal Services Centre, Chinese University of Hong Kong and raised in the Laboratory Animal Services Center, School of Chinese Medicine, Hong Kong Baptist University. The mice were raised in the Laboratory Animal Services Center, School of Chinese Medicine, Hong Kong Baptist University, in accordance with “Institutional Guidelines and Animal Ordinance” (Department of Health, Hong Kong Special Administrative Region) (Registration No. LIUYE/15-16-01-CLNC). Body weight, food consumption, and blood glucose level were monitored each week. Blood glucose level was determined by OMRON glucometer (Beijing, China) using blood samples collected from the tail vein.

**Animal Groups**

The diabetes mice model was induced by high-fat diet (adjusted Calories Diet, 42% from fat) (No. 881372) (Harlan Laboratories, Inc., Indianapolis, IN, United States) for 4 weeks and intraperitoneal (i.p) injection with STZ (25 mg/kg) three times in following 3 days. The mice with fasting glucose level higher than 11 mmol/L were considered as diabetic mice, and the diabetic mice with consecutive 7-day hyperglycemia (11 mmol/L or greater) were used for the experiment. An equal volume of vehicle was injected into the control mice. The diabetic mice were then divided into the diabetic group (model group), the CP treatment group (CP group) and glibenclamide treatment group (positive group). For the CP treatment group, the mice were orally administrated 2 g/kg/day CP until end of the experiment according to previous experimental data (Supplementary Figure S1). The normal mice were divided into two groups: vehicle control group (0.5% CMC-Na solution treated, named as blank group) and CP-treated control group (CP treated, named as control group). CP-treated control group mice were orally administered 2 g/kg/day CP extract and blank group mice were orally administered same volume 0.5% CMC-Na solution as CP solution. Glibenclamide was given at 15 mg/kg/day in 0.5% CNC-Na solution to mice in positive group according to the previous study (Xiao et al., 2017).

**Blood Sample Collection and Preparation**

For serum pharmacokinetics study, mice were orally administered CP solution and anesthesia by 3% chloral hydrate through intraperitoneal injection after 10 mins. About 1 mL blood was collected in heparin-tube. Plasma was obtained after 3,000 rpm centrifuge for 30 min at room temperature. A total of 900 μL MeOH was added to 100 μL plasma and centrifuged at 14,000 for 10 min at 4°C to precipitate protein. A total of 800 μL supernatant was dried under vacuum concentrator within 30 min and dissolved in 200 μL 70% methanol for LC-MS analysis.

For lipids study, about 1 mL blood was collected from mice under anesthesia, serum was obtained after centrifuging at 3,000 rpm for 30 min at room temperature. A total of 50 μL serum was used for lipids study. Briefly, 250 μL Folch solvent with the internal standard was added in 50 μL serum and vortexed vigorously. Two phases were formed after centrifugation at 5,000 rpm for 15 min. The bottom layer was dried under vacuum concentrator and dissolved in 200 μL of ACN/IPA/H2O (65:30:5 v/v/v) for analysis.

Another 100 μL serum was used for clinical index analysis including ALT, AST, ALP, CREA and BUN using a biochemical analyzer (Hitachi 902 Automatic Analyzer; Hitachi, Japan), TG, TCHO, LDL, and HDL were analyzed using assay kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) according to the manufacturers’ instructions.

**Hepatic Histopathology Analysis and Biochemical Analysis**

At the end of the study, the mice were sacrificed, and liver tissue was collected for H&E staining to analyses histology changes. Liver tissue from sacrificed mice was soaked in 10% formalin solution (prepared by 1× phosphate-buffered saline, PBS) and fixed for 12 h. Samples were then made into paraffin sections as specimens and stained with hematoxylin and eosin (H&E) according to manufacturer’s protocol (Mayer’s Hematoxylin Solution, Sigma-Aldrich). The sections were observed and captured under microscopy. About other 50 mg liver tissues were homogenized in iced 1× PBS solution (1:10, m/v). The homogenized solution was used for TG and TCHO analysis following the protocol of Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

**Phytochemical and Metabolomics Study**

**Phytochemical Analysis**

The chromatographic analysis was performed on an Agilent 1290 UPLC system equipped with an autosampler, binary gradient pump, and PDA detector. The system was operated at 30°C and a water ACQUITY UPLC HSS T3 column (150 mm × 2.1 mm,
The injection volume was 2 mL. The elution conditions were as follows: 0–5 min, linear gradient 2–5% B; 5–10 min, linear gradient 5–10% B; 10–15 min, linear gradient 10–25% B; 15–25 min, linear gradient 25–40% B; 25–28 min, linear gradient 40–90% B. Peaks were detected at 254 nm. 

**Lipidomics Study**

The liquid chromatogram was performed on an Agilent 1290 UPLC system. A Waters ACQUITY UPLC HSS C18 column (2.1 mm × 100 mm, 1.8 μm particle size) was used for separation. The column temperature was maintained at 40°C and autosampler temperature was maintained at 8°C. The separation was performed with mobile phase A and B within 20 min per sample. Phase A consists of 60:40 water/ACN in 10 mM ammonium formate and 0.1% formic acid, and phase B is made by 90:10 IPA/ACN with 10 mM ammonium formate and 0.1% formic acid. Positive mode and 6 L for the negative mode.

**Bioinformatics and Statistical Analysis**

The predicted targets and lipids targets of CP were combined together to upload to STRING (Szklarczyk et al., 2014) for PPIs analysis to link the action of predicted targets with lipids targets of CP.

**Results**

**CP Attenuated Diabetic Dyslipidemia in Diabetic Mice Induced by High Fat Diet and STZ**

As shown in Table 1, after high fat diet and STZ treatment, blood glucose levels of mice were significantly elevated whereas their body weight was significantly demoted compared with control and blank group, indicating that the diabetic mice model was well established. In contrast, those clinical signs were significantly rescued after CP treatment for 5 weeks. Since both hyperlipidemia and hepatic steatosis are characterized as

| TABLE 1 | Blood glucose (mM) levels and body weight changes on week 0, 3, and 5. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Blood glucose (mM)** | **Blank** | **Control** | **Model** | **CP** | **Positive** |
| **Week 0** | 6.8 ± 1.2 | 6.1 ± 0.9 | 17.0 ± 3.5 *** | 16.2 ± 4.3 | 16.5 ± 5.2 |
| **Week 3** | 6.7 ± 1.0 | 6.6 ± 1.4 | 17.5 ± 4.0 *** | 12.7 ± 3.2 * | 12.6 ± 3.5 * |
| **Week 5** | 6.0 ± 1.0 | 5.1 ± 0.5 | 16.6 ± 3.0 *** | 10.7 ± 1.5 *** | 10.4 ± 2.0 *** |
| **Body weight (g)** | **Week 0** | 23.8 ± 0.8 | 23.5 ± 1.8 | 24.4 ± 1.0 | 24.4 ± 1.0 | 24.5 ± 0.9 |
| **Week 3** | 24.6 ± 1.0 | 25.0 ± 1.0 | 23.4 ± 1.8 * | 24.1 ± 0.3 | 23.9 ± 1.3 |
| **Week 5** | 26.3 ± 1.2 | 26.2 ± 1.3 | 23.2 ± 1.8 * | 24.9 ± 0.6 * | 24.6 ± 1.2 * |

*The effects of CP extract on blood glucose levels and body weight of diabetic mice. All data are presented as means ± SD. Diabetic model group vs. control group: *p < 0.05, **p < 0.01 and ***p < 0.001. CP or glibenclamide (positive) treatment group vs. diabetic model group: *p < 0.05, **p < 0.01, ***p < 0.001.
substantial lipid disorders in diabetes, we determined serum TCHO, LDL, HDL, and TG levels, and hepatic AST, ALT, TG, and TCHO levels. Liver histopathological changes were also monitored. As shown in Figure 1 and Supplementary Figure S4, compared to that of control and blank group, serum TG, TCHO, and LDL levels, as well as hepatic AST, ALT, TG, and TCHO levels of the model group were significantly increased, whereas serum HDL levels were significantly decreased, indicating that...
FIGURE 2 | The lipidomics study on serum from diabetic mice: Metabolic profiles and multivariate data analysis. (A) PCA score plot of the blank group (red color), control group (green color), model group (dark blue), CP group (light blue), and positive group (purple). (B) PLS-DA score plot with 95% confidence interval of all studied groups. (C,D) OPLS-DA score plot and S-plot between model group and CP treatment group. (E) Heatmap of selected metabolites was built based on one-way ANOVA rankings and hierarchical clustering. Each dot represents one mouse.
the hyperlipidemia and hepatic steatosis were developed in the model group. In CP or glibenclamide-treated groups, those altered lipids-associated indexes were significantly alleviated. In addition, histological examination of hepatic sections also revealed that there were a large number of vesicles of fat accumulating within hepatocytes of diabetic mice, which was significantly reduced after CP or glibenclamide treatment. These results suggested that CP extract has a great potential to attenuate diabetic dyslipidemia and hepatic steatosis induced by high fat diet and STZ in mice.

**CP Improved Circulating Lipid Metabolism in Diabetic Dyslipidemia**

To study the CP effect on lipid metabolism in diabetic mice, we performed a lipidomics study on mice serum. Fatty acyls (fatty acids), glycerolipids (DG and TG), glycerophospholipids (PC, PE, and PI), and sphingolipids (SM) were then used for multivariate statistical analysis as shown in [Supplementary Table S1.1](#). As the PCA and PLS-DA are two effective multivariate analysis methods for large-scale data matrix, the combining data were analyzed using PCA and PLS-DA approaches. As shown in Figures 2A,B, PCA and PLS-DA score showed a clear classification between model group and control group, demonstrating that circulating lipids profiles are significantly different in the model group. As well, the CP-treated group or glibenclamide treated group also had its own distinctive classification, bearing some overlap. Considering the overlap between model group and CP-treated group, we further narrowed down the profiles of lipids in these two groups using OPLS-DA method. Notably, CE, DG, TG, PC, and SM were significantly either up-regulated or down-regulated (p < 0.05) (Figure 3). After importing those CP-regulated lipids to Cytoscape, a network pathway was built based on KEGG database and revealed that metabolic pathways including arachidonic acid metabolism, bile acid biosynthesis, de novo fatty acid biosynthesis, glycerophospholipid metabolism, glycosphingolipid metabolism, linoleate metabolism and saturated fatty acids beta-oxidation are involved in the regulating function of CP in lipid metabolism (As shown in [Supplementary Figure S5](#)).

**CP Possess Multiple Components-Multiple Targets-Multiple Pathways Properties for Diabetic Dyslipidemia**

Since absorption into the bloodstream is one of the prerequisites for drug efficacy, we performed a pharmachemistry study to detect the CP components in blood stream after oral administration in mice. As shown in Table 2 and [Supplementary Figure S2](#), after oral administration of CP to normal C57/BL6 mice, 13 compounds in total: quinic acid, neochlorogenic acid, chlorogenic acid, 4-hydroxybenzoic acid, gallic acid, quercetin-3-glucuronide, kaempferol, loganin 7-pentoside, astragalin, kaempferol-3-rhamnoside, quercetin, quadrinoside IV, and asiatic acid were detected. Subsequently, we used in-house tools "MOST: most-similar ligand-based approach to target prediction" (Huang et al., 2017) to predict the protein targets of major potential active components of CP identified in bloodstream as shown in [Supplementary Table S1.2](#). Sixty-nine

| No. | Compound     | Classification | Blank plasma | Plasma after 10 min administration | Plasma after 30 min administration |
|-----|--------------|----------------|--------------|------------------------------------|------------------------------------|
| 1   | Quinic acid  | Organic acid   | –            | ✓                                  | ✓                                  |
| 2   | Neochlorogenic acid | Organic acid | –            | ✓                                  | –                                  |
| 3   | Chlorogenic acid | Organic acid | –            | ✓                                  | ✓                                  |
| 4   | 4-Hydroxybenzoic acid | Organic acid | –            | ✓                                  | ✓                                  |
| 5   | Gallic acid  | Organic acid   | –            | ✓                                  | –                                  |
| 6   | Quercetin-3-glucuronide | Flavonoid | –            | ✓                                  | ✓                                  |
| 7   | Astragalin   | Flavonoid      | –            | ✓                                  | ✓                                  |
| 8   | Kaempferol   | Flavonoid      | –            | ✓                                  | ✓                                  |
| 9   | Logand 7-pentoside | Flavonoid | –            | ✓                                  | ✓                                  |
| 10  | Kaempferol-3-rhamnoside | Flavonoid | –            | ✓                                  | ✓                                  |
| 11  | Quercetin    | Flavonoid      | –            | ✓                                  | ✓                                  |
| 12  | Quadrnoside IV | Saponin       | –            | ✓                                  | ✓                                  |
| 13  | Asiatic acid | Saponin        | –            | ✓                                  | ✓                                  |

![Figure 2A](#) Figure 2A: PCA and PLS-DA score showed a clear classification between model group and control group. As shown in Figure 2B-E, PCA and PLS-DA score showed a clear classification between model group and control group. Notably, CE, DG, TG, PC, and SM were significantly either up-regulated or down-regulated (p < 0.05) (Figure 3). After importing those CP-regulated lipids to Cytoscape, a network pathway was built based on KEGG database and revealed that metabolic pathways including arachidonic acid metabolism, bile acid biosynthesis, de novo fatty acid biosynthesis, glycerophospholipid metabolism, glycosphingolipid metabolism, linoleate metabolism and saturated fatty acids beta-oxidation are involved in the regulating function of CP in lipid metabolism (As shown in [Supplementary Figure S5](#)).

**TABLE 2** Constituents of CP identified in plasma.
FIGURE 3 | Cyclocarya paliurus improved circulating lipids profiles in diabetic mice model. (A) Cholesterol ester (CE), (B) Diglyceride (DG); (C) Triglyceride (TG); (D) Sphingomyelin (SM); (E) Phosphatidylcholines (GP-PC); (F) Phosphatidylinositol (PI); (G) Phosphatidylethanolamine (GP-PE); (H) Lysophosphatidylethanolamine (GP-LPE). All data are presented as means ± SEM (n = 6–8). #p < 0.05, ##p < 0.01, ###p < 0.001 (comparison between control and model group); *p < 0.05, **p < 0.01, ***p < 0.001 (comparison between CP group and model group).
targets were matched for flavonoids, 59 targets were matched for organic acids, and 2 targets were matched for saponins. There targets were searched by related key words online to determine the relevance of diabetic dyslipidemia. Five targets were selected after reference searching. Finally, we linked the lipids targets with predicted targets using STRING (Szklarczyk et al., 2014) to build interaction networks of predicted targets and lipids targets via PPI. Among these compounds, the main predicted targets of CP were ALOX12, APP, BCL2, CYP2C9, and PTPN1 while the predicted-targets linked lipids targets via PPI analysis were PLA2G(s) and PI3K(s) families as shown in Supplementary Figure S3.

We then conducted reference searching to review the experiment study on these targets as shown in Supplementary Table S2. The ALOX, BCL-2, CYP 2C9, PLA2G(s) and PI3K(s) families, PLD2 and PTEN were determined as quercetin and kaempferol targets on diabetic dyslipidemia (Guo et al., 2017). PTPN1, PI3K(s) family and PLD2 were determined as targets of saponins such as quadranoside IV and asiatic acid (Ramachandran and Saravanan, 2015), whilst BCL-2, PI3K(s) family, PLD2 and PTEN were determined as action targets of gallic acid and 4-hydrobenzoic acids (Figure 4).

DISCUSSION

Hyperlipidemia is the most common form of dyslipidemia, refers to the abnormally elevated levels of any/all lipids or lipoproteins in the blood, frequently happened in long-term type II diabetes patients (Dixit et al., 2014). Recent studies have indicated that diabetic dyslipidemia may not only be the consequence but also the cause of disturbed glucose metabolism (Parhofer, 2015). Recently, CP was reported to improve insulin sensitivity (Jiang et al., 2014), attenuates inflammation (Wang Z. et al., 2017), and control hyperglycemic and hyperlipidemic abnormalities (Xu et al., 2017) both in vitro and in vivo, although the hypolipidemic mechanism has not been elucidated yet. In this study, we confirmed CP alleviated lipid dysfunction in diabetes, particularly diabetic dyslipidemia, as revealed by the clinical index, histological analysis, and lipidomics analysis. Mechanistically, we employed a network pharmacology approach to determine that CP’s hypolipidemic effect involvement in PI3K and MAPK signaling pathways.

Lipidomics is a powerful tool to investigate lipid profiles changes. Previous studies indicated that circulating lipidomes were correlated with hyperlipidemia and hepatic steatosis.
MAPK can influence the expression of CYP2C9 (Bachleda et al., 2006; Zhang et al., 2011; Zhao et al., 2011), and activation of PI3K alters expression of PI3K signaling pathway (Rahmani et al., 2013; Sugiyama et al., 2017). ALDH2, ALOX15, and BCL2 and PI3K were predicted as direct targets of CP, whilst PLD2, PTEN, and PI3K were validated experimentally. Further experimental work can be performed to uncover the relationship between targets-targets. Next, the potential active compounds we identified in this study have been shown to be effective for anti-diabetic studies on animals, the systemic evaluation of combinatorial use on these compounds have not been conducted yet. Additional in vitro studies in future will help to uncover the multiple pharmacological mechanisms found in herbal medicines.

CONCLUSION

In this study, we report CP attenuated diabetic dyslipidemia and hepatic steatosis in high fat diet and STZ-induced diabetic mice. The lipidomics study revealed CP improves circulatory lipid disorder, and the serum pharmacology study revealed quinic acid, neochlorogenic acid, chlorogenic acid, 4-hydroxybenzoic acid, gallic acid, quercetin-3-glucuronide, kaempferol, loganin 7-pentose, astragalin, kaempferol-3-rhamnoside, quercetin, quadinoside IV, and asiatic acid are potential active components of CP. Combining lipidomics and bioinformatics analysis, ALOX12, APP, BCL2, CYP2C9, and PTEN1 were predicted as direct targets of CP, whilst PLD2, PTEN and PL2A2G(s) and PI3K(s) families were predicted as lipids linked targets of CP in diabetic hyperlipidemia. In conclusion, the CP was shown to be a multi-component and multi-targets herbal product with potent lipid regulation properties in dyslipidemia.

AUTHOR CONTRIBUTIONS

Z-xB and H-tX designed the study and revised the manuscript. Ht-X, BW, C-hL, LxZ, and Z-wN performed the animal
experiment. LxZ performed the clinical index analysis, lipidomics analysis, bioinformatics analysis, and wrote the manuscript. Z-w WN performed phytochemicals analysis of CP. TH performed bioinformatics analysis and revised the manuscript. LZ and C-y L provided technical support and advices toward study.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2018.00973/full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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